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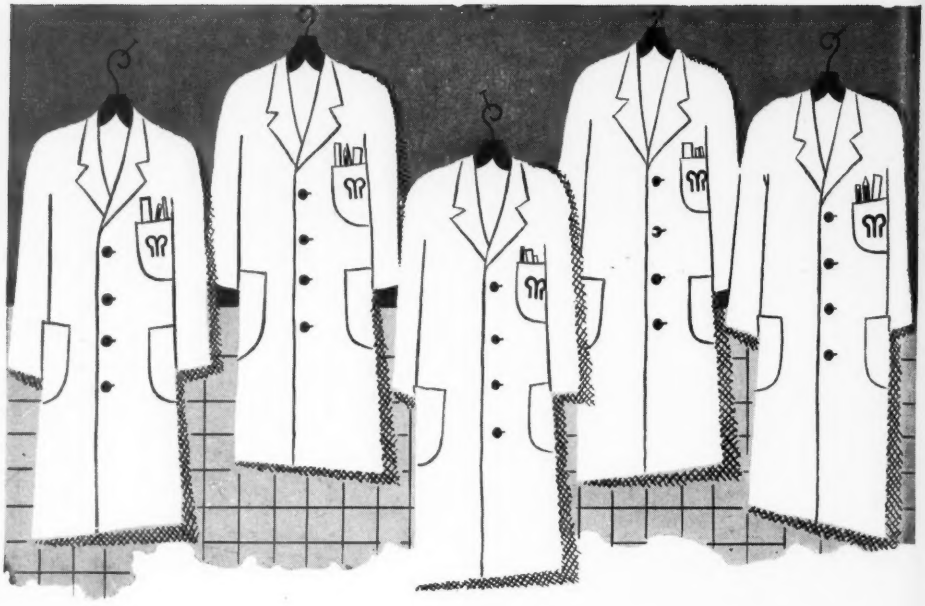

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URINARY CORTICOSTEROID VALUES OF CHILDREN AS DETERMINED CHEMICALLY

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IT HAS been demonstrated that the urine of normal adult persons contains material which has biologic activity similar to that of the glycogenic hormones of the adrenal cortex. This urinary material increases under various conditions of stress and of stimulation of the adrenal cortex with adrenocorticotropic hormone. It decreases in amount when the function of the adrenal cortex is impaired. Several chemical methods for the determination of this material have been based on the assumption that it has chemical properties similar to or identical with those of the known cortical hormones. However, none of the chemical methods is entirely specific and all yield values which are much greater than those obtained by bioassay. Nevertheless, the substances determined chemically also fluctuate in some measure as the activity of the adrenal cortex changes. It is probable that the values obtained with some of the chemical methods bear a relation to the biologic activity which is analogous to the relation of the neutral 17-ketosteroids to the androgenic activity of the urine. Various names have been given to the urinary material under discussion. The term "corticosteroids" seems appropriate since the evidence indicates that the material is produced by the adrenal cortex and that its chemical properties are similar to those of adrenal cortical hormones.

Inasmuch as few determinations of urinary corticosteroids of children have been made, the present study was undertaken with the following con-

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* Fellow in Medicine.

siderations in view: 1) determination of the distribution of values according to age, 2) individual variations and 3) variations following stress.

METHODS

Urine was collected in glass bottles without preservative during twenty-four-hour periods. The parents, and the children, when old enough, were carefully instructed as to the necessity for an accurate collection of the urine. They were questioned afterward, and if there was any doubt as to the adequacy of the collection, that specimen was discarded. The urine was acidified to pH 1 immediately after completion of the collection, allowed to stand overnight at room temperature, and then extracted with chloroform within one to three days.

The method for determination of corticosteroids was essentially that described by Corcoran and Page (1). This chemical method is based on the reaction of periodic acid (HIO_4) with α-ketols or α-diols having a primary alcohol group to yield a molecule of formaldehyde. The formaldehyde liberated is determined colorimetrically with the aid of chromotropic acid. The values of the urinary corticosteroids obtained in this study are expressed as milligrams of 11-dehydrocorticosterone. Although the substances measured will be designated "corticosteroids," "formaldehydogenic substances" would be a more accurate term. Probably, in addition to corticosteroids, other substances of unknown origin are present in urine which yield formaldehyde in the procedure used.

Several studies were made on premature infants, newborn babies and children less than 1 year of age. Their urine was collected by absorption with cotton, and care was taken to avoid contamination with feces. An approximately equal volume of 0.1 N hydrochloric acid was added to the cotton and this mixture was allowed to stand for one to three days. The water was removed by filtration with suction and the cotton was extracted twice with 1 volume of chloroform. The aqueous portion was further extracted with chloroform and the chloroform extracts were combined and assayed. As a control, cotton without urine was extracted in like manner to test the possibility that impurities were present which would interfere with the color reaction. The cotton used in this study did not yield any extractives which interfered with the procedure.

CLINICAL MATERIAL

Group 1.—This group consisted of 95 children who were living at home, with the exception of the newborn babies and 2 others, and who were without clinical manifestation of disease.[1] There were 32 boys and 63 girls.

[1] Two children, each 1 year old, were hospitalized. Each had suffered from poliomyelitis and was living in the hospital while receiving physical therapy.

Their ages ranged from 2 days through 15 years. This group was studied to establish the average values for urinary corticosteroids during childhood, and to note the effect of age and individual variabilities on the values obtained. From these children, 121 twenty-four-hour specimens were collected and assayed.

Group 2.—From this group of 14 hospitalized[2] children, 68 twenty-four-hour specimens of urine were collected for assay. The purpose of this study

TABLE 1. VALUES OF URINARY CORTICOSTEROIDS OF CHILDREN AND ADULTS WITHOUT DISEASE, IN RELATION TO AGE

Age, years	Subjects	Determinations	Corticosteroids	
			Mean, mg./24 hrs.	Range, mg./24 hrs.
Newborn	7	7	0.18	0.09–0.26
1		2	0.24	0.22–0.26
2		11	0.23	0.14–0.42
3		7	0.24	0.17–0.31
4		5	0.24	0.13–0.31
5		8	0.31	0.16–0.52
6		5	0.24	0.18–0.29
7		10	0.26	0.17–0.44
8		8	0.22	0.19–0.26
9		5	0.25	0.19–0.33
10		9	0.36	0.26–0.50
11		5	0.29	0.26–0.35
12		16	0.30	0.23–0.38
13		6	0.32	0.27–0.40
14		10	0.32	0.20–0.51
15		7	0.45	0.16–0.85
Adults				
Male	19	19	0.52	0.21–1.16
Female	12	19	0.60	0.44–0.79

was to evaluate the effects of stress (trauma, surgical procedures and infections) on the urinary excretion of corticosteroids by children.

RESULTS

Group 1.—Table 1 shows the average and range of values for each age group of the children, and for comparison, the average and range of values obtained from determinations made on 38 twenty-four-hour urine specimens collected from 19 adult men and 12 adult women.

Three premature babies and 4 newborn babies are included as one age

[2] One child (Fig. 5, Case 6) was not hospitalized.

group. Although the values obtained from the premature group tended to be lower than the values obtained from the newborn group, the number of determinations is too small to make the observed differences significant. Because there is an unequal number of boys and girls in each age group, statistically it cannot be shown whether there is a difference in the amount excreted by either sex. However, as shown in Figure 1, there appears to

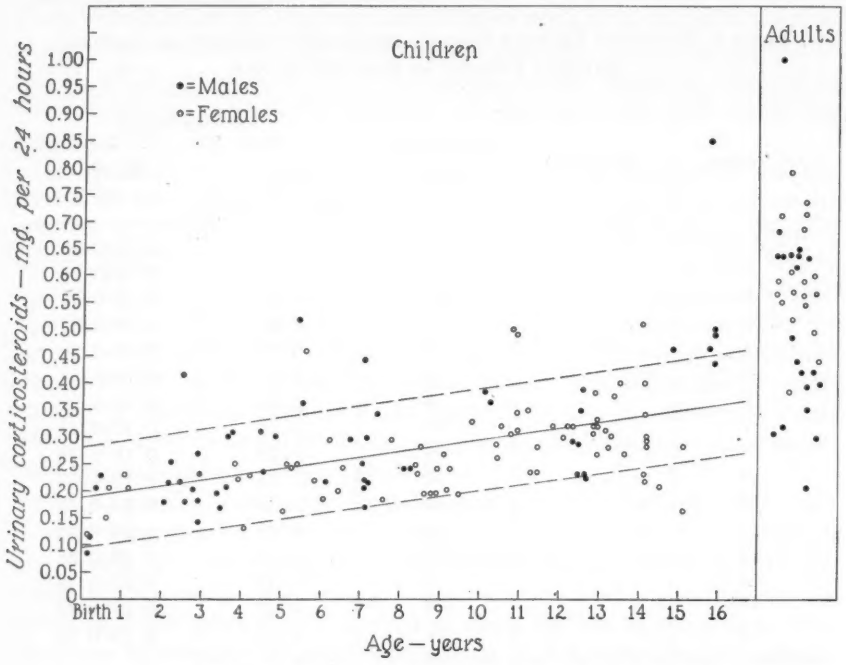

FIG. 1. Values of urinary corticosteroids of children and adults without disease, in relation to age.

be a tendency for boys to excrete a larger amount than girls in any age group; but the adult values show no statistically significant difference in the amount excreted by either sex. Therefore, one may surmise that there appears to be a tendency for urinary corticosteroids to reach adult values in boys sooner than in girls.

The milligrams of urinary corticosteroids in twenty-four hours are plotted as the ordinate against the chronologic age as the abscissa in Figure 1. The left portion of the chart shows the individual values obtained from boys (black circles) and girls (white circles) less than 16 years of age. The extreme right side of the chart shows the individual values obtained from

adult men and women. The solid line is the regression line and the distance between the two broken lines represents two standard deviations. The regression line indicates an increase of 0.01 mg. of urinary corticosteroids for each yearly increase in age. Twenty, or 16 per cent, of the 121 determinations differ from the mean by more than two standard deviations. Although statistically there is an increase of 0.01 mg. of urinary cortico-

TABLE 2. VALUES OF URINARY CORTICOSTEROIDS OF CHILDREN AS DETERMINED BY DIFFERENT METHODS

Author	Children	Sex	Age	Corticosteriods	
				Determined by chemical method, mg. per 24 hrs.	Determined by biologic method, glycogen or "E" units*
Venning and Kazmin (2)	6	M	1– 4 days		0.0
	4	M	3– 8 days	0–0.2	
Day (3) (method of Talbot)	1	M	2– 3 days	0.05	
	2	M	6– 8 weeks	0.06–0.1	
Matson and Longwell (5) (method of Heard, Sobel and Venning)	8†	M	3–33 days	0.17–0.38	
	5‡	M	5–17 days	0.26–0.62	
Heard, Sobel and Venning (6)	1	M	2½ years	0.32	34
	1	M	3 years	0.47	42
	1	M	5½ years	0.7	53
	1	M	7 years	0.79	58

* Glycogen unit = E unit = biologic activity equivalent to that of 1 microgram of 17-hydroxy-11-dehydrocorticosterone.

† Premature infants.

‡ Full-term infants.

steroids for each yearly increase in age, there is marked overlapping of values obtained from year to year as noted in Table 1. After the tenth year of life the values appear more consistently to approach adult values.

Table 2 summarizes the few results obtained from the literature. Venning and Kazmin (2), using a biologic method, and Day (3), using a chemical method, found that newborn babies excreted very small amounts of urinary corticosteroids. Our values are comparable to those of Day. The method used in this study would be expected to give somewhat higher values than the method of Talbot and his associates (4), which was used

by Day, since this latter method measures only those reducing substances extractable from benzene by water.

Recently Matson and Longwell (5) reported the results obtained from 13 infants, using the method of Heard, Sobel and Venning (6). These workers found appreciable amounts of corticosteroids in both the premature and newborn infants' urine. Their values were somewhat higher than those obtained in this study, but the method of Heard, Sobel and Venning has consistently yielded higher values.

By means of the glycogen deposition method, Venning and Kazmin, and Heard and his associates found that boys of 5 and 7 years of age excreted

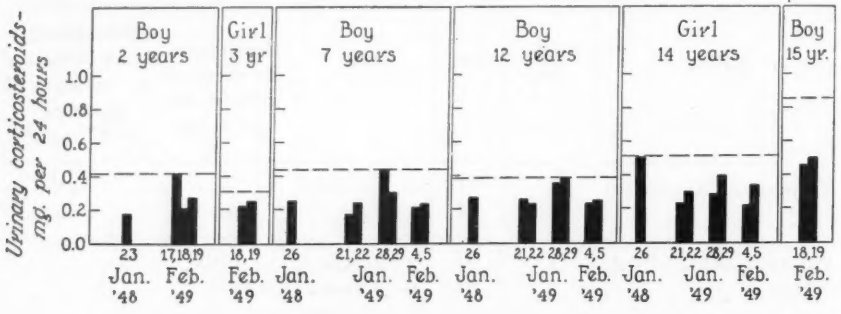

FIG. 2. Individual variations of urinary corticosteroids. The horizontal broken line indicates the upper range of normal for that particular age.

amounts of urinary corticosteroids which approached adult values, whereas by use of a chemical method, the latter authors found that children 5 and 7 years of age excreted less than adults. As determined in the present study by a different chemical method, there is a gradual rise in the urinary corticosteroids, which approach low adult values after the tenth year of life. The discrepancy between the results of chemical and biologic assay has been interpreted to mean that the biologic activity of the steroids excreted reaches adult values before the amount of steroids detected by chemical methods.

Talbot (7) recently has concluded that the values of urinary corticosteroids obtained from children and adults vary directly with the body surface area and that there is little if any difference between children and adults in the amount excreted per square meter of body surface area. This relationship will be considered by our associates in a subsequent paper.

Daily variations of the excretion of urinary corticosteroids of 6 children of different ages are presented in Figure 2. The greatest variation for any one individual was approximately ± 30 per cent of the mean for that individual. Talbot and co-workers (8) found from a study of the day-by-day

output of 3 normal men and 1 normal woman that the chances were two out of three that the value for a single day would fall within ±30 per cent of the average value for that individual. Heard, Sobel and Venning, and Venning and Kazmin found individual variations of the same magnitude.

Group 2.—Weil and Browne (9), Venning, Hoffman and Browne (10) and Shipley and co-workers (11) have reported that a rise in the excretion

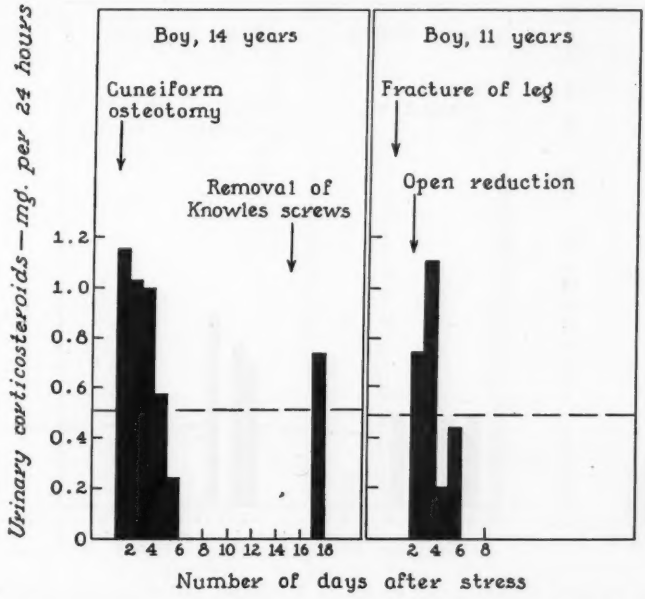

FIG. 3. Urinary corticosteroid values of 2 children following orthopedic procedures (Cases 1 and 2). The horizontal broken line indicates the upper range of normal for that particular age.

of urinary corticosteroids by adults follows stress and that those substances tend to remain elevated until convalescence is practically completed. Studies of urinary corticosteroids of children following stress have not been reported. Figures 3, 4, 5, 6 and 7 show graphically examples of the values of urinary corticosteroids obtained from children suffering from various types of stress (trauma, surgical procedures, infections). The horizontal broken line indicates the upper range of normal values determined for that particular age and the arrow indicates the day the stress occurred.

The data obtained from 2 children (Cases 1 and 2) who had undergone orthopedic operations are shown in Figure 3. The values obtained immediately after operation rose to a peak which was approximately 2.3

times the upper range of normal for the respective ages. These values tended to return to the normal range by the third to fifth postoperative day.

The values for 2 children (Cases 3 and 4) presented in Figure 4 show similar responses to another type of surgical treatment. The first child (Case 3) suffered from familial hemolytic icterus and underwent splen-

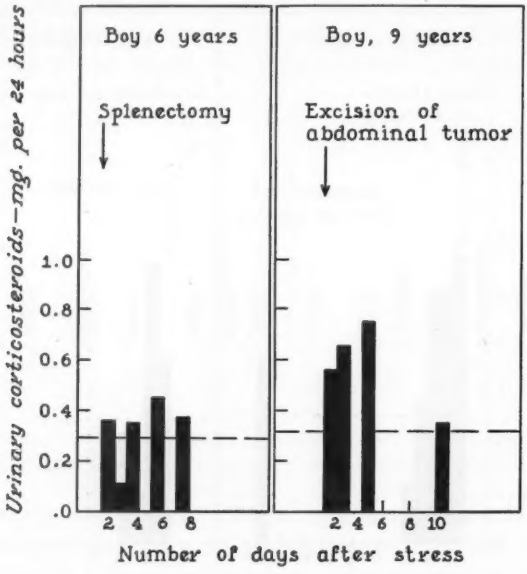

FIG. 4. Urinary corticosteroid values of 2 children following laparotomy (Cases 3 and 4). The horizontal broken line indicates the upper range of normal for that particular age.

ectomy. Four postoperative urine specimens contained only moderately increased amounts of corticosteroids. The second child (Case 4) underwent the more extensive abdominal surgical treatment included in excision of a retroperitoneal ganglioneuroblastoma. He responded with a peak output that was more than twice the highest value for healthy children of this age. The values were considerably greater than those found in the case of the child who underwent splenectomy. The peak value did not reach the maximal absolute values obtained from the orthopedic patients, but the per cent increase over the highest normal value was about the same. Clinically, the children who underwent splenectomy and resection of the ganglioneuroblastoma appeared to be as ill as the children who underwent orthopedic operations.

Figure 5 (Cases 5 and 6) shows the urinary corticosteroid excretion of 2 children who were subjected to the stress of fever or infection or both. The boy of 11 years of age (Case 5) suffered from a cold and fever and had a convulsion three days before the first urinary corticosteroid value was determined. This value was somewhat higher than normal for his age. At this time his temperature was 102° F. Seventeen days later the value

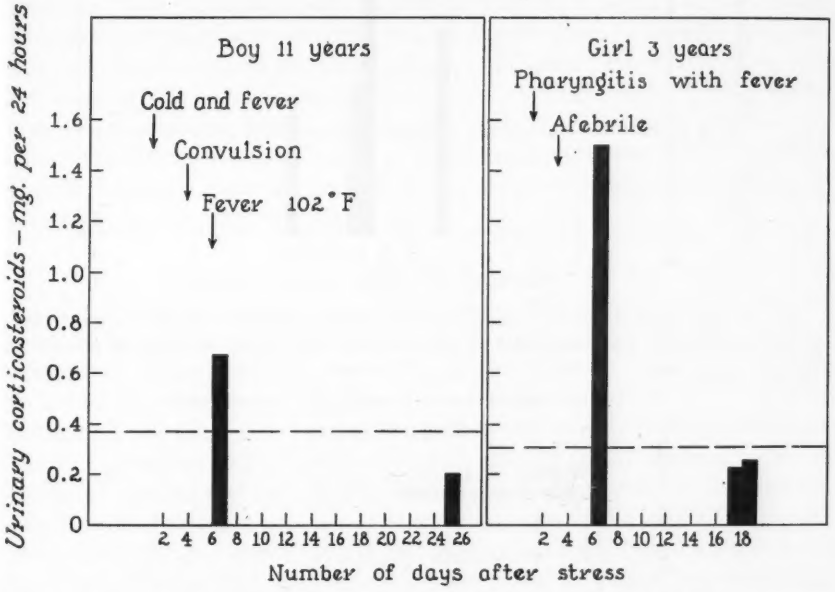

FIG. 5. Urinary corticosteroid values of 2 children following acute infections (Cases 5 and 6). The horizontal broken line indicates the upper range of normal for that particular age.

for the urinary corticosteroids was within normal limits and he was clinically well. The 3-year-old girl (Case 6) had had pharyngitis six days before the excretion of corticosteroids was determined and had been afebrile for four days. Nevertheless, she excreted almost five times the maximal normal amount for her age, although clinically she was cured at the time the determination was made.

Forbes and co-workers (12) in a study of the 17-ketosteroids in trauma and disease, found that in certain debilitated patients an alarm was not followed by a rise of 17-ketosteroids. They concluded that this lack of response might be due to a previous alarm, and that the patients were already in the stage of resistance or even approaching the stage of exhaus-

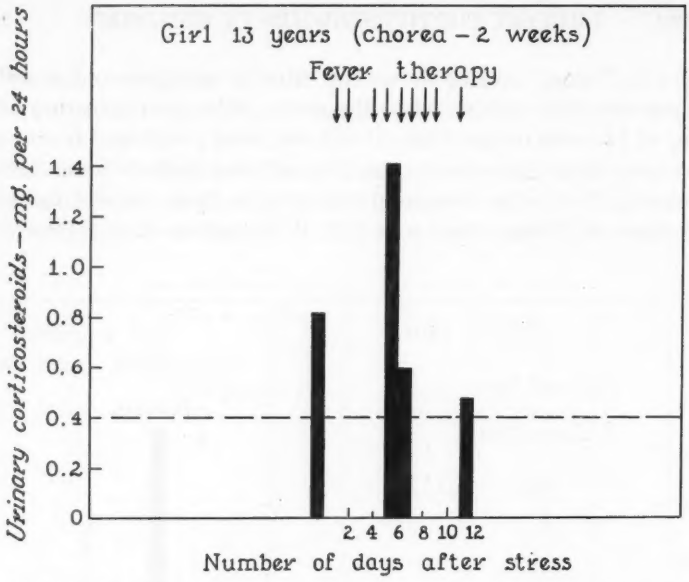

FIG. 6. Values for urinary corticosteroids of a child suffering from chorea and treated with fever therapy. The horizontal broken line indicates the upper range of normal for that particular age.

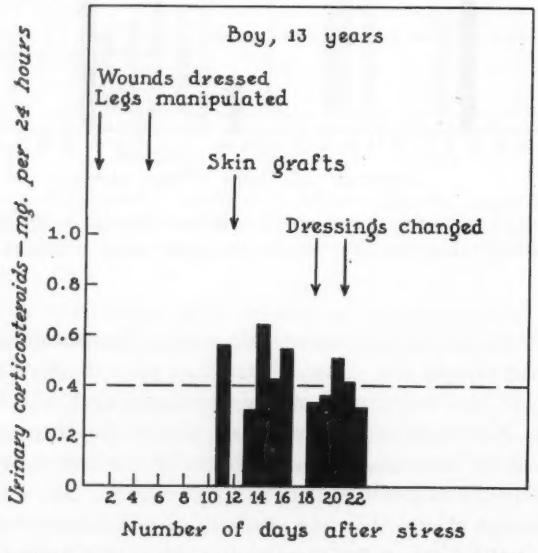

FIG. 7. Values for urinary corticosteroids of a child suffering from infected burns, who received frequently applied stresses of different types; that is, manipulation of legs, skin grafts and anesthesia. The horizontal broken line indicates the upper range of normal for that particular age.

tion and that therefore the cortex was apparently unable to respond to further stimuli. Figures 6 and 7 represent the effect on the urinary corticosteroids of stress applied to subjects already suffering from disease. The 13-year-old girl (Fig. 6, Case 7) suffered from chorea for two weeks before admission to the hospital. The first value obtained was about twice the maximal normal value for her age. After five days of fever therapy it was increased to about three and one-half times the maximal normal level. However, two subsequent determinations showed a decline in the values although fever therapy was continued. In Case 8 (Fig. 7) the child suffered from infected burns and flexion deformities of the legs. He received frequent dressings of the wounds, manipulations of the legs while he was under anesthesia, and skin grafts. In this case there does not appear to be any correlation between the incidence of stress and an increase in the excretion of corticosteroids although some of the corticosteroid values are significantly above the maximal normal level.

COMMENT

It has been our experience that the urine of untreated patients suffering from Addison's disease often contains small amounts of formaldehydogenic substances. Heard and his associates (6) also found some lipide-soluble reducing substances in the urine in cases of panhypopituitarism even though glycogenic activity could not be detected. Consequently there may be some doubt as to the significance with respect to the adrenal cortex of the small amounts of formaldehydogenic substances found in the urine of newly born children, particularly since Venning and Kazmin (2) were unable to detect glycogenic activity in the urine of children 1 to 4 days of age. Nevertheless, the values obtained are useful as a base line for evaluation of possible abnormalities in the amounts excreted in pathologic conditions. This last statement also is valid for the values determined for older children.

Venning and Kazmin found glycogenic activity in the urine of male children $2\frac{1}{2}$ to 3 years of age, and after the age of $5\frac{1}{2}$ years they found adult male values for this activity. The values for the formaldehydogenic material increase gradually with age and approach the adult range as the child approaches adolescence. It is believed that this increase of urinary output is indicative of an increased production of adrenal cortical steroids. The gradual increase of urinary 17-ketosteroids supports the idea that the adrenal cortex produces more steroids as the child grows older.

The response of the urinary corticosteroids to stress in children was similar to the response observed in adults. Although the number of cases was too small to permit definite conclusions, orthopedic surgical procedures and infections appeared to result in the greatest increases of urinary corti-

costeroids. There was no correlation between the clinical condition of the child and the amount of corticosteroids present in the urine. The children with chronic infections responded to additional stress with a smaller increase of urinary corticosteroids than other children subjected to a similar stress. However, in view of variables, such as metabolism of the adrenal hormones, the effects of which are unknown, it would not be justifiable to conclude from measurements of excretory products alone that the adrenal cortices of the children with infections were capable of less response to the stimulus of stress than the cortices of children without infection.

SUMMARY

The values for corticosteroids obtained by 121 determinations on twenty-four-hour specimens of urine from 95 children are presented. This group included 32 healthy boys and 63 healthy girls. Their ages ranged from less than 1 year through 15 years. Values for the urinary corticosteroids increased by 0.01 mg. for each increase of 1 year in age. The values overlapped in contiguous age groups and varied in different individuals of the same age group, and the values for the older age groups tended to overlap the values for adults. Measurable amounts of corticosteroids appeared in the urine of premature and newborn infants. The children responded to stress (trauma, surgical procedures and infections) by excretion of increased amounts of urinary corticosteroids.

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FAILURE OF HYPOPHYSEAL GROWTH HORMONE TO PRODUCE NITROGEN STORAGE IN A GIRL WITH HYPOPHYSEAL DWARFISM*†

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THE isolation of the hypophyseal growth hormone in a pure form by Li, Evans and Simpson (1) has made possible the use of the purified hormone in clinical experiments. This pure hormone was first administered in the spring of 1947 to a girl judged clinically to be a hypopituitary dwarf. At a dose level approaching 5 milligrams per kilogram of body weight it was without effect upon nitrogen balance. It is the purpose of this paper to report these balance studies: the complete long-term study of the patient which includes the administration of growth hormone for four months will be reported subsequently.

CASE PRESENTATION AND METHOD OF STUDY

The patient (F. H.) was a girl, aged 16½ years, with a bone age of 13 years and a dental age of 12 years. She weighed 70½ lbs. (32 Kg.), was 49.8 inches (127 cm.) in height, and had an I.Q. of 130. Her early development was said to have been normal; retarded growth was first apparent at the age of 5. After the age of 12 years she had grown approximately one inch in height, no gain in height having occurred in the two and one-half years prior to this study. Previous therapy with thyroid by mouth, injections of crude anterior pituitary extracts, and implantation of two calves' anterior pituitaries into her broad ligament had been without effect upon her growth rate.

On physical examination she was notable primarily for her small stature, her alert mentality, her prepubertal external genitalia and secondary sex characters, and the absence of axillary and pubic hair. The pertinent laboratory and X-ray findings included the following: sella of normal size, BMR of −22 per cent, blood cholesterol of 254 mg. per cent, normal glucose tolerance and insulin tolerance curves, serum calcium 9.1 mg. per cent, serum phosphorus 4.1 mg. per cent, 24-hour 17-ketosteroid excretion less

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* The Anterior Hypophyseal Growth Hormone used in this study was produced by two of the authors, Doctors Choh Hao Li, and Herbert M. Evans, who were aided by a special grant from Wesley W. West of Houston, Texas, to The Institute of Experimental Biology.

† The authors gratefully acknowledge the assistance of Miss Virginia Stokdyk who supervised preparation of the diet, and of Mrs. Ruth Lerner who carried out or supervised the calcium and phosphorus determinations.

than 1 mg., and 24-hour urinary gonadotropin excretion less than 3 mouse units. In spite of her low basal metabolic rate and high blood cholesterol it was thought that her failure to respond to exogenous thyroid, plus her high I.Q., excluded primary hypothyroidism. Her configuration was not that typical of ovarian aplasia, and an episode of acute appendicitis made possible the inspection of her ovaries. They were infantile in character, and did not resemble those seen in the syndrome of ovarian aplasia.

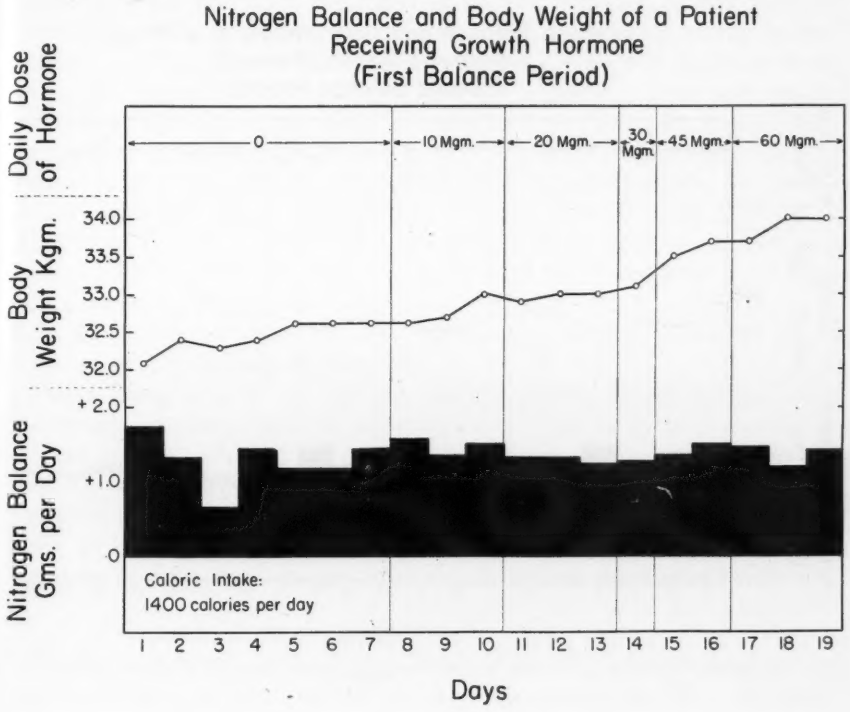

FIGURE 1

During the balance periods, the patient was given a formula diet made up of whole milk, skimmed milk powder, salt, eggs, sugar and cream. During the first balance period the diet provided approximately 1400 calories, with 66 Gm. of protein per day. During the second balance period the food intake was reduced to approximately 1150 calories with 51 Gm. of protein per day. The daily intake of diet was weighed to an accuracy of ±4 Gm., and aliquots from each lot of diet were analyzed for nitrogen, calcium and phosphorus. Urine was collected daily and stools were collected and pooled for periods varying from four to nine days. The analyses for nitrogen, calcium, and phosphorus respectively were done by the micro Kjeldahl procedure, the method of Larson and Greenberg (2), and the method of Fiske and Subbarow (3).

OBSERVATION AND COMMENT

In Figures 1 and 2 are presented the data for nitrogen balance of the two periods of metabolic study. A positive nitrogen balance is represented by a black column above the zero line, and a negative nitrogen balance is represented by a diagonally hatched column below the zero line. It will be noted that during the first balance period, when the patient was receiv-

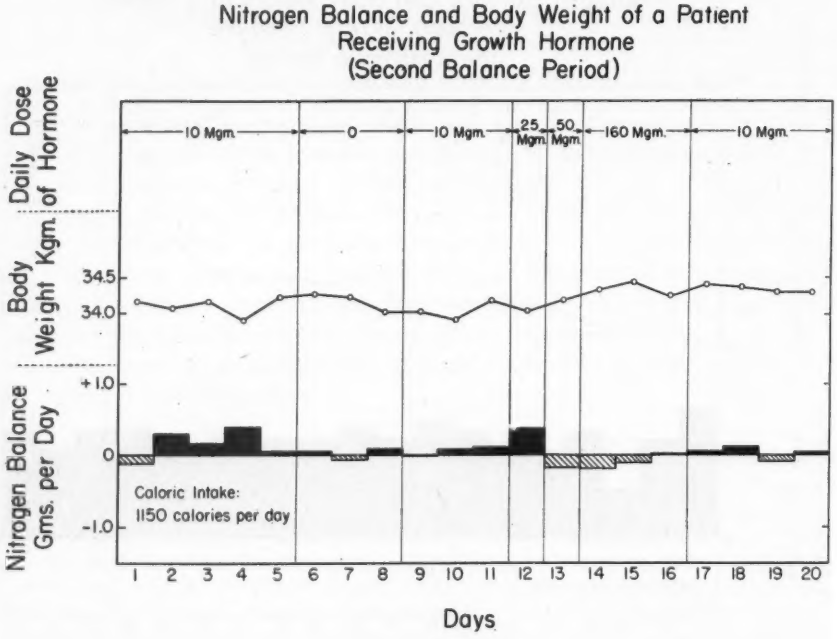

FIGURE 2

ing the larger caloric intake, she gained weight steadily and was in a positive nitrogen balance throughout the entire period. The degree of positive nitrogen balance was in no way influenced by institution of growth hormone therapy, nor by increasing the daily dose of growth hormone to 60 mg. In the second balance period, when the caloric intake was less, the patient maintained a steady weight and was essentially in nitrogen equilibrium throughout the entire period. Increasing the daily dose of growth hormone up to 160 mg. did not alter this equilibrium. During the first balance period when growth hormone was given in doses of 45 and 60 mg. per day, there was an apparent retention of calcium and phosphorus. In view of the fact that a similar retention did not occur in the second balance period, it was judged not to be significant. In neither balance period were

significant changes in blood glucose, NPN, serum calcium, phosphorus, or phosphatase observed.

It is worthy of comment that the patient reacted with local urticarial formation and pruritus to an intradermal inoculation of 0.05 cc. of a solution containing 0.1 mg. of growth hormone per cc. (before she received the first subcutaneous injection of growth hormone). This raised the question of whether she had been sensitized to growth hormone by her previous treatment with crude extract or/and the implantation of the calves' pituitaries. After desensitization she tolerated the growth hormone injections well except for local soreness with the larger injections and the development of urticaria and giant urticaria on days 13 and 14, respectively, of the first balance period. These urticarial reactions were controlled by benadryl. She was afebrile throughout both balance periods, except for minor afternoon temperature rises to 37.4° C., 37.4° C., and 37.6° C. on days 9, 13 and 15 of the first balance period, and afternoon temperature rises to 37.3° C., 37.7° C., and 37.4° C. on days 18, 19 and 20 of the second balance period. There seems little likelihood that in this patient the anabolic effect of growth hormone could have been masked by increased catabolism secondary to a febrile response.

After completion of the second balance period, blood samples were taken and an effort made to demonstrate in vitro or in vivo antibodies against growth hormone. It was impossible in vitro to demonstrate precipitins. The admixture of 2 cc. of the patient's serum with 0.015 mg. of growth hormone (from the same bottle from which the patient received 160 mg.) prior to injection did not alter the in vivo effectiveness of the growth hormone in producing nitrogen retention in hypophysectomized rats.

SUMMARY

During two different metabolic balance periods, growth hormone in doses varying from 10 to 160 mg. per day was administered to a 16½-year-old patient with hypophyseal dwarfism and a bone age of 13 years. No effects on calcium, phosphorus, or nitrogen balance were observed. Attempts to demonstrate the presence of antibodies to growth hormone or anti-growth hormone effects of the patient's serum were unsuccessful.

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THE QUANTITATIVE DETERMINATION OF URINARY PREGNANEDIOL IN PREGNANT WOMEN: THE INFLUENCE OF THE METHOD UPON RESULTS

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THE first procedure described for the quantitative determination of urinary pregnanediol, the principal excretory product of progesterone, was that of Venning (1) which depends upon the extraction, purification and gravimetric measurement of pregnanediol glucuronide as the sodium salt (NaPG). Although this method was carefully worked out by its originator from a quantitative aspect and has given curves of excretion that appear to be of physiologic significance in late pregnancy, it is open to a number of objections, even when applied to pregnancy urine of high pregnanediol titer, and has been found to be of limited value in early pregnancy and in the nonpregnant woman in whom the amount of pregnanediol excreted is below the level of quantitative accuracy or specificity of the method. Other methods commonly used since 1941 are based on the procedure developed by Astwood and Jones (2). This utilizes the fact that pregnanediol, freed from the glucuronide conjugate by hydrolysis, may be precipitated out of 95 per cent ethyl alcohol by the addition of 4 volumes of water. As originally described, the purified precipitate was weighed. The method was rendered more sensitive by Talbot *et al.* (3) who, in the final measurement of the purified pregnanediol, took advantage of the strong yellow color which this compound yields with concentrated sulfuric acid.

Neither Astwood and Jones nor Talbot *et al.* were able to get very satisfactory recovery of pregnanediol or sodium pregnanediol glucuronide added to urine, by their procedure. Nor could any consistent correction factor for loss with reprecipitation, as was worked out by Venning for her method, be assumed. Sommerville, Gough and Marrian (4) subjected the "Astwood Talbot" method to very careful study. By standardizing the precipitation process as to time and temperature they were able to get consistent recoveries of 90 to 100 per cent of added pregnanediol glucuronide when as little as 1 mg. (calculated as pregnanediol) was present. Even with only 0.4 mg. of added pregnanediol, in the form of the glucuronide, the recovery by their modified "Astwood-Talbot" procedure was 70 to 80 per cent.

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With their modification, therefore, the method would appear to be highly accurate so far as recovery of small amounts of urinary pregnanediol is concerned. The fact that blank determinations on 12 different male urines yielded only traces of apparent pregnanediol (no more than 0.385 mg./24-hour volume) argued well for the specificity of the method. The authors warned, however, that overestimates of considerable magnitude might be obtained if the procedure were applied to "pathological urines containing abnormally high concentrations of 17-ketosteroids or of other neutral steroids of adrenal origin."

The object of the present communication is, first, to report our experience with the Sommerville, Gough and Marrian procedure, during which we have gained evidence that it is prone to yield gross overestimates in the urines of pregnant women and in the occasional urine from apparently normal nonpregnant individuals, both male and female. Secondly, we shall report results of a comparison between values acquired by a modified Sommerville, Gough and Marrian procedure which removes nonspecific chromogens and those acquired by the Venning method on aliquots of urines collected at various stages of pregnancy. These comparative figures are of interest in view of the present realization that compounds other than pregnanediol glucuronide may be recovered by the Venning method.

EXPERIMENTAL

Before applying the method of Sommerville *et al.*, to the determination of urinary pregnanediol in pregnant women we decided to test our technical skill with this unfamiliar procedure by performing a recovery experiment. A "washed toluene" urine blank was prepared from a pooled collection from 7 normal young men, to aliquots of which pure pregnane-3(α), 20α-diol (melting point 238 to 240°C.) was added. In the process of these determinations we were considerably disturbed by the marked turbidity of the supernatant fluid after centrifuging the precipitates. This phenomenon was noted even after the third precipitation and applied to the urine blank determinations as well as the recovery experiments. The final recovery amounted to 147 to 150 per cent of the amounts added, if the value of the blank determinations was not allowed for. Subtracting the blank, which amounted to 1.26 mg./100 cc. of urine, the recovery was 96 to 98 per cent.

Because of the high titer of this particular male urine blank, a phenomenon not encountered by Sommerville *et al.*, nor by us to such a degree in subsequent specimens, we decided to experiment with methods of removing this nonspecific chromogenic material. The most successful of these experiments were those in which the precipitated "pregnanediol" was collected on a sintered glass funnel and washed with petroleum ether. By this means the apparent pregnanediol of this particular urine blank was reduced to 0.06 mg./100 cc. of urine, the turbidity of the supernatant fluid after reprecipitation was eliminated, and the recovery of added pregnanediol was still excellent—97 to 99 per cent, after subtracting the value of the urine blank.

As a result of this experience considerable work has been done with recovery experiments and comparative assays of both pregnancy and non-

TABLE 1. COMPARATIVE ASSAYS OF PREGNANEDIOL IN THE URINES OF PREGNANT WOMEN

(Representative results)

Urine specimen	Pregnanediol, mg. per 24-hour volume			
	Recovered as NaPG	Free—according to Sommerville *et al.*		
	According to Venning*	Unmodified, Method A	Petroleum ether wash	
			Of 1st ppt., Method B	Of 4th ppt., Method C
T—32 wks.	56.0 (55.1)†	64.0	46.5	47.5
F—19 wks.	25.7	29.6	16.8	16.5
F—29 wks.	65.0	56.2	54.5	55.1
F—34 wks.	91.0	148 (153)	74.5	75.1
V—15 wks.	19.2	13.1	10.4	10.8
V—28 wks.	36.0 (34.8)	29.5 (28.2)	27.8	27.9
V—28 wks. and 1 day	35.2 (34.5)	38.5 (39.1)	26.8	27.2
D—25 wks.	46.5	49.0	41.3	42.5
D—32 wks.	62.5	52.0	49.6	49.5
C—19 wks.	22.7	21.2	15.6	16.1

* In recovery experiments in this laboratory, with recrystallized NaPG added to male urine, Dr. Venning's corrections for loss with a second precipitation in water and acetone have been checked. The actual NaPG content of the urine, therefore, is calculated on the basis of her figures. Her conversion factor of NaPG to pregnanediol, however, is not applicable under the atmospheric conditions in our laboratory. Precipitates collected at various times of year have been dried for twelve hours in vacuo over P_2O_5 at 125 to 130°C. They have all been found to lose 9.5 per cent of their weight and to regain this amount when again exposed to atmospheric conditions. On this basis, NaPG in our laboratory takes up 3 molecules of water instead of the 1 molecule reported by Venning. Our conversion factor, therefore, is 0.557 rather than 0.597.

† Figures in parentheses represent duplicate determinations.

pregnancy urines, with and without the petroleum ether wash. In the first place it was ascertained that as little as 1 mg. of pure pregnanediol precipitated out of 4 cc. of alcohol with 16 cc. of H_2O could be collected on a sintered glass funnel of medium porosity, washed 3 times with 10 cc. of petroleum ether and redissolved in hot alcohol with no loss (4 experiments with 97 to 101 per cent recoveries). It was next found, in the analysis of urine, that only 1 additional precipitation out of alcohol was necessary if the first precipitate was washed with petroleum ether. Such values agreed very closely with those acquired when the final alcohol extract obtained by the unmodified Sommerville, Gough and Marrian method was subjected to a

fourth precipitation and washed with petroleum ether. The former will be referred to as Method B, the latter as Method C, and the unmodified procedure, involving 3 precipitations with no petroleum ether wash, as Method A. All 3 of these methods as well as the Venning procedure have been performed upon aliquots of 35 urine specimens from 5 pregnant women. Representative results are given in Table 1 and show the close agreement between Methods B and C. Since Method B, involving only 2 precipitations, is considerably less time consuming it has been the one finally adopted. The details of this procedure are as follows:

A portion of a 24-hour urine specimen expected to contain about 1 to 5 mg. of pregnanediol is hydrolyzed in the presence of $\frac{1}{5}$ of its volume of redistilled toluene, extracted twice more with toluene and the toluene extract washed twice with normal NaOH and twice with water exactly as described by Sommerville *et al.*, the only difference being that the toluene emulsions, instead of being passed through a Büchner funnel, are centrifuged. After centrifugation we have found that the clear toluene and clear urine may be decanted off of a mat of black residue directly into a clean separatory funnel and the urine layer drawn off and added to the rest of the residual urine for re-extraction. By centrifuging after each extraction and using the same centrifuge tube throughout, the black residue gets well washed with toluene. The clear toluene extract, after being washed with NaOH and water, is evaporated completely to dryness, using an all-glass vacuum still, and quantitatively transferred with 95 per cent ethyl alcohol (refluxed over NaOH pellets and redistilled twice) to a 50 cc. conical centrifuge tube which has a short side arm about 1 inch from the top and is graduated at 4 cc. A Folin anti-bumping tube is placed in the centrifuge tube, the contents of which are evaporated down to 4 cc. by placing in a beaker of boiling water. The time and temperature conditions for precipitation as described by Sommerville *et al.* are strictly adhered to, the anti-bumping tube being used as a stirring rod. We do not add Hyflo-Super-Cel, since its presence interferes with visualization of the precipitate and since we also found that it did not help much in preventing the precipitate from "floating" after centrifugation. After the first precipitation with N/10 NaOH has incubated overnight (leaving the anti-bumping tube in it) the precipitate is collected with suction on a sintered glass funnel of medium porosity using the anti-bumping tube as a pouring rod. The centrifuge tube and precipitate are then washed through 3 or 4 times with 5 cc. of hot (75°C.) 1:4 ethanol-N/10 NaOH solution (v/v), 3 times with 5 cc. of 1:4 ethanol-H_2O (v/v), and finally twice with 10 cc. of petroleum ether. The anti-bumping tube is then carefully placed on a rack, the sintered glass funnel connected to the original 50 cc. centrifuge tube into which the pregnanediol is collected by washing through twice with 4 cc. and 3 times with 2 cc. of boiling 95 per cent ethanol, the suction tube being attached to the side arm. The anti-bumping tube is then replaced in the centrifuge tube, the contents of which are evaporated down to 4 cc. The second precipitation is carried out exactly like the first except that 16 cc. of distilled H_2O (rather than N/10 NaOH) is added (drop by drop over a 3-minute interval at 75°C with constant stirring). After slow cooling and a 2-hour incubation period, the anti-bumping tube is washed down with 1 cc. of the 1:4 solution of ethanol-H_2O and carefully placed on a rack. After centrifuging for 15 minutes at 3500 r.p.m., the supernatant fluid is decanted off of the precipitate. Whether or not the filter-aid is used, this precipitate will sometimes float. With the aid of the anti-bumping tube, the supernatant liquid is therefore poured through a Whatman No. 4 filter paper (washed free of steroids with

TABLE 2. RECOVERY OF PREGNANEDIOL ADDED TO URINE BLANKS, USING METHOD B (PETROLEUM ETHER WASH)

Expt. #	Urine specimen		Pregnanediol added to an equal volume or extract thereof, (mg.)	Recovery of pregnanediol (corrected for blank)	
	Donor, volume used and point at which pregnanediol was added	Apparent pregnanediol content of urine blank, (mg.)		(mg.)	(%)
1	H. F.—male—150 cc. unextracted urine	0.039	2.0 2.0	1.84 1.92	92 96
2	G. S.—male—100 cc. washed toluene extr.	0.010	2.0 2.0	1.98 1.94	99 97
3	O. S.—female—surg. castrate—200 cc. toluene extr. before washing with NaOH	0.003	1.0 1.0	0.95 0.97	95 97
4	A. M.—male—150 cc. unextracted urine	0.010	1.0 1.0	1.01 0.98	101 98
5	H. F.—male—150 cc. washed toluene extr.	0.089	0.5 0.5	0.42 0.44	84 88
6	O. S.—female—surg. castrate—200 cc. unextracted urine	0.003	0.5 0.5	0.40 0.41	80 82
7	O. S.—female surg. castrate—200 cc. unextracted urine	0.002	2.17 } added as re- 2.17 } crystallized NaPG	2.08 2.11	96 97

alcohol and ether) taking pains that as much as possible of the precipitate is left in the centrifuge tube. Two cc. of the 1:4 ethanol-H_2O mixture is pipetted onto the filter paper, washing down the sides so that any small particles will be collected at the base of the funnel. The funnel is then placed in the 50 cc. centrifuge tube (no need to remove the anti-bumping tube) and washed through twice with 2 cc. of boiling 95 per cent ethanol. The funnel is then placed in a test tube graduated at 20 cc. and long enough so that the tip of the funnel is well above the graduation mark. Another cc. of ethanol is used to wash down the sides of the centrifuge tube. The precipitate, now dissolved in 5 cc. of ethanol, is treated with Norite exactly as described by Sommerville *et al.*, and quantitatively transferred with hot 95 per cent ethanol through the same filter paper into the graduated tube. It is cooled, made to volume with 95 per cent ethanol, and an aliquot, judged to contain 0.1 to 0.5 mg. of pregnanediol is transferred to a tube graduated at 10 cc. After

evaporating off the alcohol in a water bath the tube is left for several hours in a vacuum desiccator over $CaCl_2$. Concentrated H_2SO_4 (A. R.) is run in from a fast pipette or burette exactly to the 10 cc. mark and stirred with a glass rod. The tube is placed in a water bath maintained at 25°C. for 20 minutes, its contents being stirred vigorously at 5-minute intervals. It is then poured into a colorimeter tube for assay. A calibration curve with pure pregnane 3(α), 20α-diol is run for each group of unknowns. We use a Coleman, Jr. spectrophotometer set at a wave length of 450λ and read off of the scale for optical density. The readings are made at 35 to 40 minutes from the time of adding the H_2SO_4.

Recovery experiments

From the recovery experiments reported in Table 2, one may conclude that this method involves no more than a 5 per cent loss of pure pregnanediol[1] added either to unextracted urine or to the toluene extracts, provided as much as 1 mg. is present. In recovery experiment No. 7 a sample of recrystallized[2] NaPG was added to the urine of a surgical castrate. The excellent recovery checks the observations of Sommerville *et al.*, in which the method of hydrolysis and extraction as well as the precipitation procedure were shown to give quantitative yields of pregnanediol added as the glucuronide. It appears that the use of the sintered glass funnel and the petroleum ether wash, as described, introduce no added chance of loss.

As is shown by the results in Table 1, the introduction of the petroleum ether wash makes it possible to reduce the number of precipitations from 3 to 2 without any greater chance for overestimations. It has been ascertained, however, that neither the second precipitation nor the Norite treatment of Method B may be omitted.

Gravimetric versus colorimetric assay

The possibility was investigated that gravimetric measurement could be substituted for the somewhat nonspecific color reaction of pregnanediol with H_2SO_4. In the unmodified Sommerville, Gough and Marrian procedure (Method A) a large amount of perfectly white material is recovered from some urines which gives very much greater overestimates by weight than by the colorimetric assay. Since this was particularly true of urines in which the turbidity of the supernatant fluid after the third precipitation was marked, and since this turbidity was almost entirely eliminated by washing the first precipitate with petroleum ether, it was thought that gravimetric measurement might be feasible in Method B. Experiments

[1] Pregnane 3(α), 20 α-diol prepared from pregnancy urine by Methods B and C and recrystallized out of 95 per cent alcohol to a constant melting point of 244 to 245°C.

[2] This NaPG was recovered from the urine of a woman just before labor. Upon recrystallization out of 95 per cent alcohol it melted at 281 to 282°C. with decomposition and evolution of gas. It gave an essentially negative reaction for ketosteroids when the Zimmermann reagents were added to 2.5 mg.

both with urine blanks and with aliquots of pregnancy urines, however, demonstrated that gravimetric assay results in very much higher values than are given by colorimetric assay (Table 3). Since the procedures used have been shown to give a quantitative recovery of pure pregnanediol, these high values with gravimetric determinations must represent gross overestimations. As may be seen from the table, this applies even in late pregnancy when relatively large amounts of pregnanediol are recovered from relatively small volumes of urine. We concluded, therefore, that colorimetric assay renders the method not only more sensitive, but very much more specific.

TABLE 3. COMPARISON OF RESULTS BY GRAVIMETRIC VERSUS COLORIMETRIC ASSAY OF PREGNANEDIOL IN URINE

Urine specimen		Method A (No petroleum ether)		Method B (Petroleum ether wash)	
Donor	Vol. used	Gravimetric	Colorimetric	Gravimetric	Colorimetric
Nonpregnancy		Pregnanediol, mg. per volume extracted			
7 males, pooled specimen	100 cc.	18.6	1.26 (1.28)*	1.60	0.06 (0.05)
O. S.—female, surg. castrate	150 cc.	3.2	0.01	0.95	0.01
Pregnancy		Pregnanediol, mg. per 24-hour volume			
F—18 wks.	150 cc.	27.5	12.6	23.0	12.3
F—19 wks.	150 cc.	106.0	29.6	29.3	16.8
F—20 wks.	300 cc.	87.5	24.7	25.2	17.9
K—31 wks.	100 cc.	98.5	70.0	72.2	48.7
K—31 wks. +1 d.	100 cc.	86.0	60.0	65.7	49.1
T—24 wks.	100 cc.	47.5	26.8	38.7	24.6
F—29½ wks.	50 cc.	92.0	81.0	89.5 (86.0)	69.5 (70.1)
F—30 wks.	50 cc.	126.0	88.5	100.0 (96.5)	78.0 (82.5)
V—15½ wks.	100 cc.		15.1	14.0	12.6 (11.8)
V—17½ wks.	100 cc.		17.6	26.5 (29.8)	14.1 (14.8)

* Figures in parentheses represent duplicate determinations.

Characteristics of the petroleum ether soluble material in pregnancy urine

In order to gain some idea of the nature of the nonspecific chromogens removed by petroleum ether, the petroleum ether washings from 71 determinations by Method B or C upon the urines of 6 pregnant women were pooled. This pool represented 13 liters of urine, equivalent to 8½ 24-hour

volumes. The collections were distributed over the tenth to the thirty-sixth weeks of pregnancy. The weight of the dry material, a brown oil with some visible white crystals, was 44.1 mg. Its total chromogenic activity with concentrated H_2SO_4 was equivalent to that of 30.5 mg. of pure pregnanediol, although the color produced was brownish rather than the pure yellow of pregnanediol. Eighty-seven per cent of this "apparent" pregnanediol was not precipitated by digitonin.[3] The Leiberman-Burchard color reaction on the digitonide precipitates, when read against a cholesterol standardization curve, was calculated as 2.1 mg. of cholesterol to 44.1 mg. of the pool. Since the chromogenic activity of cholesterol with H_2SO_4, according to our findings, is less than half that of pregnanediol, this amount would have contributed very little of the color produced by the petroleum ether wash. Neither the digitonizable fraction nor the supernatant gave the deep red color typical of 17-ketosteroids with the Zimmermann reagents, but the supernatant fraction gave a purple color similar to that of 20-ketosteroids. This may have been pregnane-3(α)-ol-20-one, which, according to Sommerville *et al.* (4) is not entirely removed even by 3 precipitations but which is so weakly chromogenic with H_2SO_4 that it would contribute very little to the "apparent" pregnanediol. Read off of a 20-ketosteroid calibration curve and calculated as pregnanolone it amounted to 4.7 mg. to 44.1 mg. of the pool.

No further work has as yet been done to identify the nonspecific chromogens of pregnancy urine removed by petroleum ether. Since 87 per cent of this chromogenic material is not precipitated with digitonin, we are apparently not dealing either with cholesterol or with the nonpregnanediol compound recently described by Guterman and Kraus (7) which is found in male urine after muscular exertion and which is recovered and measured by their modification of the Astwood-Talbot procedure.

Comparative assays in pregnancy urine

Upon 105 specimens collected between the tenth and thirty-sixth weeks of pregnancy in 6 women we have performed pregnanediol determinations by 3 methods: the Venning, the unmodified method of Sommerville *et al.* (Method A) and our modification of the latter using the petroleum ether wash (Method B).[4] The corrections for loss of NaPG with reprecipitation

[3] Aliquots equivalent to 1.76 mg. of the pool were digitonized according to the method of Sperry (5) for serum cholesterol. The supernatants and the precipitates were treated according to the quantitative technic of Frame (6) for separating 3α- and 3β-hydroxysteroids. The H_2SO_4 color tests were run upon aliquots of both fractions, 87 per cent of the chromogenic activity of the untreated petroleum ether wash being found in the supernatant and only 5 per cent in the 3β-hydroxy fraction freed from the digitonin precipitate with pyridine. Duplicate determinations gave check results.

[4] The initial object of this study was to throw further light upon the controversial subject of the effect of diethylstilbestrol administration to pregnant women upon preg-

and the conversion factor for pregnanediol recovered as NaPG have been checked under the conditions of this laboratory (*see* footnote, Table 1). From the representative results shown in Table 1, it is apparent that overestimates of considerable magnitude are frequently acquired by Method A when applied to the urine of pregnancy. In many specimens this procedure gives values as high or even higher than those acquired by the Venning method in which the pregnanediol is measured as a glucuronide. It is now known that at least 1 constituent other than pregnanediol (pregnane-3(α)-ol-20-one) is recovered as a glucuronide from pregnancy urine and must give overestimates in the measurement of pregnanediol as NaPG (Marrian and Gough (8)). The results from Method B, therefore, with which complete recovery of pure pregnanediol has been demonstrated, must be very much closer to the actual pregnanediol content. (It should be emphasized, however, that we have no proof that petroleum ether wash removes *all* nonspecific chromogens.) Of particular importance is the observation that the degree of overestimation by Method A varies over a very wide range (0 to 76 per cent higher than Method B) and shows marked fluctuations in the same individual.[5] Examples of the latter are shown in analyses on consecutive days in subject V (Table 1), and subject K (Table 3). Because of this, the curve of excretion by Method B is very much smoother than by Method A. (This is particularly true when stilbestrol is being given, an observation that will be enlarged upon in a subsequent communication. The start of stilbestrol therapy or an increase in dosage temporarily depresses the excretion of the nonspecific chromogens.)

Comparative assays in nonpregnancy urine

Although most of our work with pregnanediol determinations has been

nanediol excretion. These 6 women were followed at various stages of pregnancy with and without diethylstilbestrol therapy. In discussing the results of comparative assays in the present communication, we are omitting those periods of observation when stilbestrol therapy (or its omission) was apparently influencing the findings under consideration. These results will be published elsewhere, as will also our conclusive evidence that stilbestrol glucuronide is not recovered by the Venning procedure.

[5] Although the amount of chromogen removed by petroleum ether varied considerably in the same individual, it was noted that the average overestimate by the unmodified procedure (Method A) was very much greater in 3 of these patients than it was in the other 3. Thus in subject T, the overestimate in 9 specimens ranged from 9 to 53 per cent, with an average of 32 per cent. In subject F (19 specimens) the range was 0 to 76 per cent, with an average of 30 per cent, and in subject K (12 specimens) the average was 34 per cent (range 8 to 57 per cent). Subjects V, D, and C, on the other hand, from whom 30, 12 and 23 specimens were analyzed, had average overestimates by the unmodified procedure of only 10, 7 and 9 per cent respectively with a range of 0 to 48 per cent. The amount of nonspecific chromogen could not be consistently related to the period of gestation, the clinical condition of the patient or the sex of the infant.

TABLE 4. COMPARATIVE ASSAYS OF PREGNANEDIOL IN THE URINES OF NONPREGNANT INDIVIDUALS

(Representative results)

Urine specimen	Condition of subject	Pregnanediol, mg. per 24-hour volume	
		Unmodified method of Sommerville *et al.*, (Method A)	Present modification with petroleum ether wash, (Method B)
G.—female 2/26–27/49	2 mo. amenorrhea, ? pregnant. Biopsy 2/30/49 = proliferative endometrium	2.59 (2.46)*	0.860 (0.858)
S.—female 5/22–23/49 6/18–19/49	Sterility problem; 7 wks. amenorrhea; atrophic endometrium. Days 16–18 of cycle, secretory endometrium	0.267 9.10 (9.15)	0.157 7.10 (6.98)
R.C.—female 2/ 3– 4/49 2/10–11/49 2/14–15/49 2/18–20/49	Last period 12/10/48 8 wks.; staining. 9 wks.; staining. 9½ wks.; staining. abortion 2/16/49	5.00 (5.22) 6.10 (5.90) 5.28 (5.25) 0.340	5.10 (5.12) 3.12 (3.82) 2.36 (2.40) 0.298
D.—female 6/28–29/49	Dysfunctional flow, proliferative endometrium	0.620	0.190
C.—female 7/15–16/49	Dysfunctional flow, atrophic endometrium	0.453	0.250
O. S.—female	Surgical castrate	0.067	0.065
H. F.—male	Aged 31, normal	1.35	0.450

* Figures in parentheses represent duplicate determinations.

upon the urine of pregnant women, a few specimens from nonpregnant individuals have been analyzed both by the Sommerville, Gough and Marrian method and by our modification (Method B) in which a petroleum ether wash is introduced. The results in Table 4 demonstrate that although there is often no very great difference between the results by the two procedures, Method A sometimes gives rather large overestimates in nonpregnant as it does in pregnant individuals. Thus, in the case of subject G, the "apparent" pregnanediol by the unmodified procedure would not have eliminated the possibility of pregnancy, whereas with Method B

there was no question of the diagnosis. In subject R. C. the pregnanediol excretion, as measured by the unmodified method, did not change significantly during the ten days of study, whereas the steadily dropping pregnanediol by Method B gave good indication of the inevitable spontaneous abortion. The unusual amount of apparent pregnanediol in the urine of H. F., a perfectly normal male, was found in 3 different specimens from this individual when the unmodified procedure was used, but was largely eliminated by the petroleum ether wash. We have not encountered it in any of 3 other males whose 24-hour urines have been analyzed.

The non-pregnanediol material of NaPG in pregnancy urine

Evidence has been accumulating that glucuronides other than that of pregnanediol may be recovered by the Venning procedure. From hydrolyzed NaPG prepared from the urine of pregnant women, Marrian and Gough (8) have isolated and identified pregnane-3(α)-ol-20-one. They found that this compound, presumably as a sodium salt of the glucuronide, could not be removed from the NaPG by repeated recrystallizations and constituted about 20 per cent of the NaPG complex. Venning (9) has presented evidence that pregnanolones are excreted in considerable and increasing amounts after the twentieth week of pregnancy. Dorfman *et al.* (10) have demonstrated that pregnane-3(α)-ol-20-one is an excretory product of progesterone. In their experiment it constituted about 20 per cent of the "apparent" pregnanediol recovered as NaPG from the urine of a male to whom progesterone was administered.

No other glucuronides have as yet been identified in the NaPG complex as recovered by the Venning procedure from the urine of normally pregnant women. Certain of the adrenal steroids, however, if present in unusual amounts, would probably be recovered by this method. This realization constitutes a serious theoretic objection to the Venning method.

A more practical objection is the fact that the method calls for extreme quantitative accuracy on the part of an experienced and well trained technician. Furthermore, "clean" crystals of NaPG cannot always be recovered, especially prior to the twenty-fifth week of pregnancy when relatively large amounts of urine must be extracted. Melting points are of little help, since pure sodium-pregnanediol-glucuronide melts at a very high temperature (282 to 283°C., with decomposition), and a considerable amount of visible impurity may be present in a sample without greatly depressing the melting point. After about the twenty-fifth week, however, unless "pregnanediol" excretion is abnormally low, at least 15 mg. of "clean" crystals of NaPG may usually be recovered from no more than $\frac{1}{3}$ of a 24-hour volume. We have considered values for "pregnanediol" as NaPG to be truly quantitative only under these conditions.

Another objection to the measurement of pregnanediol as a glucuronide is the possibility of hydrolysis prior to extraction. This danger may be entirely eliminated, according to our experience, by having the collection bottle kept in the refrigerator or in a pan of ice during the period of collection and until an hour or so before extraction. (It should be at room temperature when extracted.) It is our habit to extract on the day that the collection is completed, but repeat determinations three to five days later on specimens that have been constantly refrigerated have always given check values (± 5 per cent).

Utilizing these precautions and calculating all 24-hour volumes on the basis of a constant 24-hour output of creatinine, we have been able to get, after the twenty-fifth week

of pregnancy, extremely uniform curves of "pregnanediol" excretion of apparent physiologic significance with the Venning procedure. In our hands over the past ten years, the weekly determination of "pregnanediol" as NaPG in problem patients after the twenty-fifth week has proven of great value as a means of prognosis and an aid to the obstetrician in the clinical management of the case. No patient whose pregnanediol excretion failed to follow the normal pattern over a 4-week period has gone to term uneventfully, and no patient with a normal curve of excretion has developed toxemia, delivered prematurely or had intrauterine death of the fetus. We are inclined to believe, therefore, that whatever is measured by the Venning method in later pregnancy is of physiologic significance so far as progesterone metabolism is concerned.

If we assume that our modification of the Sommerville, Gough and Marrian procedure (Method B) gives a quantitatively accurate value for pregnanediol only, then the difference between this and that acquired by the Venning method should give the amount of non-pregnanediol material recovered as NaPG. In Table 5, we have summarized this phase of the investigation.

TABLE 5. "NON-PREGNANEDIOL" MATERIAL RECOVERED AS NaPG

Period of pregnancy (weeks)	No. of subjects	No. of specimens of urine	Range of values for "pregnanediol" as NaPG, (mg./24 hrs.)	Per cent of "pregnanediol" as NaPG not accounted for by "true" (?) pregnanediol (Method B), (Average $\pm\sigma$ m)
10–15	3	15	6.3–19.2	37 ±11
15–20	3	9	16.1–25.7	40 ±16
20–25	3	16	18.4–39.5	27 ±14
25–30	6	27	29.0–90.2	19 ± 7
30–35	6	23	37.0–92.5	20 ± 7
Last 7 days before labor	3	5	35.8–86.0	1.4 ± 0.9

In specimens collected between the tenth and twentieth weeks there was a considerable fluctuation in the degree of difference, but the percentage of "pregnanediol" as NaPG not accounted for by "true" pregnanediol was high in most specimens, with an average of 40 per cent. According to Venning (9), pregnanolones are not excreted until after the twentieth week. This marked difference may, therefore, simply reflect the greatest drawback to the Venning method, namely that "clean" crystals of NaPG are not recovered when large amounts of urine have to be extracted. (In some instances, however, the difference was large even when 15 to 20 mg. of well crystallized NaPG with no visible impurity was recovered.)

From the twenty-fifth to the thirty-fifth weeks of pregnancy, on the other hand, the NaPG crystals were practically always "clean" and the

difference in results between the two methods was considerably more uniform, amounting to about 15 to 30 per cent (average = 19.5 ± 7 per cent) of the "pregnanediol" recovered as NaPG. Twenty per cent is the approximate figure given both by Marrian and Gough (8) and by Dorfman (10) as constituting the proportion of sodium pregnane-3-(α)-ol-20-one glucuronide present in NaPG, and which, according to Marrian and Gough, cannot be removed by repeated recrystallizations.

Due to the relative insolubility of NaPG in absolute alcohol, we have as yet been unable to devise a method which is quantitatively accurate for applying the Zimmermann test for ketones to individual specimens of NaPG. Roughly quantitative tests have indicated, however, that "clean" crystals from urines of late pregnancy have an amount of ketonic material, calculated as pregnanolone, which approximates the difference in results between the two methods. This difference, therefore, may perhaps be assumed to provide a gauge of the amount of pregnanolone present in NaPG from urines collected in the latter part of pregnancy.

Of the 6 patients studied, 1 had her pregnancy artificially terminated at thirty-six weeks, 1 had an intrauterine death of the fetus, 3 spontaneously delivered prematurely and the sixth is still undelivered. Five specimens were collected during the last seven days before delivery from the 3 patients who went into labor spontaneously. In these 5 specimens the two methods gave practically identical values for pregnanediol and in 3 of the 5 the Zimmermann test gave essentially negative results. If further study confirms this evidence for a marked drop in pregnanolone excretion prior to labor, we may find that the separate determination of pregnanediol and pregnanolone will be as informative as the separate determination of estradiol, estrone and estriol has proven to be. Until a method shall have been devised for the direct quantitative determination of urinary pregnanolone, however, this cannot be accomplished. Until that time it seems probable that the Venning method, which measures both of the known excretory products of progesterone, may give values of greater physiologic significance in later pregnancy than are acquired by the determination of pregnanediol alone.

SUMMARY

It has been found that some "non-pregnanediol" material is recovered from human urine by the Sommerville, Gough and Marrian modification of the "Astwood-Talbot" method for determining urinary pregnanediol. The excretion of this material during pregnancy varies in different individuals and also shows large fluctuations in the same individual, resulting in overestimates up to 76 per cent. A modification is described which removes "non-pregnanediol" material and with which as little as 1.0 mg. of pure

pregnanediol or sodium pregnanediol glucuronide added to urine may be recovered with an accuracy of 95 to 101 per cent.

It has also been found that colorimetric assay with concentrated sulfuric acid, when applied to pregnanediol recovered from urine and purified either by this method or the unmodified procedure of Sommerville *et al.*, is very much more specific than gravimetric assay.

Comparative assays on aliquots of 105 urine specimens collected at various stages of pregnancy have revealed that the "apparent" pregnanediol recovered as the glucuronide by the Venning method is considerably greater than the amount accounted for by the measurement of "freed" pregnanediol by the present modification. In early pregnancy the difference between the two fluctuates considerably but is high in most specimens. The average difference up to twenty weeks is 40 per cent of the "pregnanediol" recovered as the glucuronide. This is probably accounted for by the difficulty in getting "clean" crystals of the latter when large amounts of urine are extracted. After the twenty-fifth week, however, the difference between the two is very much more uniform, with an average difference which amounts to 20 per cent of the "pregnanediol" recovered as the glucuronide and which may be due to the recovery of pregnanolone glucuronide by the Venning procedure.

Suggestive evidence is presented that the drop in urinary "pregnanediol" recovered as the glucuronide prior to the onset of labor may be due to a pronounced drop in the excretion of pregnanolone.

Acknowledgment

The author wishes to acknowledge the technical assistance of Mrs. Muriel Mann.

Addendum

Since this article was submitted for publication, the modified method described herein for measuring pregnanediol in hydrolyzed urine has been used rather extensively in nonpregnancy urines. When large volumes are being extracted, one frequently encounters a blue-black material which interferes with the accuracy of results. This pigment is relatively insoluble in 95 per cent alcohol. After evaporating the toluene extract of the urine to dryness therefore, we now quantitatively transfer it with portions of cold alcohol through a No. 4 filter paper into a graduated tube, make to volume, and run duplicate determinations upon aliquots of the filtrate. Practically all of the dark pigment is left behind on the filter paper.

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BASAL TEMPERATURE STUDIES IN THE AGED FEMALE: INFLUENCE OF ESTROGEN, PROGESTERONE, AND ANDROGEN

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AN ANALYSIS of basal temperature curves has become almost routine in the diagnosis and treatment of many gynecologic disorders. The practical significance of the progestational temperature rise in the normal menstrual cycle has long been appreciated and recently the work of Palmer (1), Buxton and Atkinson (2), and Davis and Fugo (3) has shed considerable light on the mechanism by which this phenomenon occurs. It is with the hope of further contributing to the general knowledge of the subject that the following observations made on patients receiving known amounts of various hormone preparations are presented.[1] These studies suggest that the regulation of the temperature curves of menstruation and gestation may depend not so much upon the individual levels of estrogen or progesterone but rather upon the estrogen-progesterone ratio at critical points during the cycles. In addition, some information concerning the influence of cyclic testosterone on the basal temperature curve is presented for consideration.

METHOD OF STUDY AND RESULTS

The individuals on whom these observations were made comprise a group of 8 women between the ages of 64 and 82 years. Initially, these patients were made to bleed periodically by estrogen exhibition and subsequent withdrawal. Once withdrawal bleeding was established in this manner, the patients were maintained on a dosage of one milligram of estradiol benzoate twice a week. Monthly cyclic shedding of the endometrium was then brought about by the administration and withdrawal of varying dosages of progesterone. The method of uterine stimulation and endometrial reactivation in the aged female by the cyclic administration of the female sex hormones has been described in detail in a previous publication (4).

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[1] The Schering Corporation has generously supplied all of the estradiol benzoate, progesterone, and testosterone propionate used in the project of sex hormone replacement in the aged female.

Basal rectal temperatures were recorded on these patients daily for a period of approximately two years. Two hundred and twenty-eight artificially induced menstrual cycles were observed. No definite pattern of temperature rise correlating with the artificially induced progestational phase of the cycle could be established, regardless of the amount or the duration of progesterone administration. It was then decided that the one milligram injection of estradiol benzoate which ordinarily immediately preceded the series of progesterone injections would be omitted, thus simulating the physiologic drop in estrogen seen subsequent to ovulation

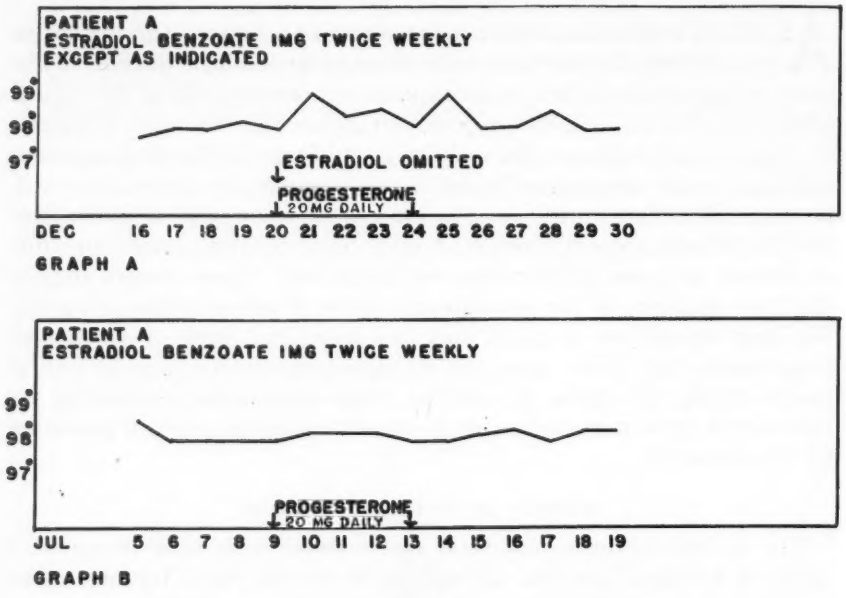

FIGURE 1

and the rise of the progesterone influence in the normal menstrual cycle. During this regimen it was noted that the patients showed a rise in temperature, which slowly returned to normal when the progesterone injections were discontinued. Thirty-two such cycles were observed.

Figure 1 shows two temperature curves (Graph A and Graph B) of the same individual. Graph A, a curve obtained after the omission of one injection of estradiol benzoate prior to the initiation of progesterone therapy, demonstrates a definite rise in temperature corresponding to the period during which progesterone was given. Graph B, obtained without the omission of an injection of estrogen prior to the onset of the particular period of progesterone therapy, shows no such rise. The progesterone dosage was

identical in both instances. Figure 2 (Graphs A and B) demonstrates the same type of response in another member of the group.

In the course of an experimental program with aged females, which was designed to investigate the effects of androgens on the estrogen-stimulated uterus, a second group of patients was studied in regard to temperature curves (5). This group consisted of 7 women from 73 to 83 years of age. Initially, each patient was given 2.0 milligrams of estradiol benzoate weekly intramuscularly in divided doses and caused to bleed vaginally by the periodic withdrawal of this hormone. When a well stimulated and responsive

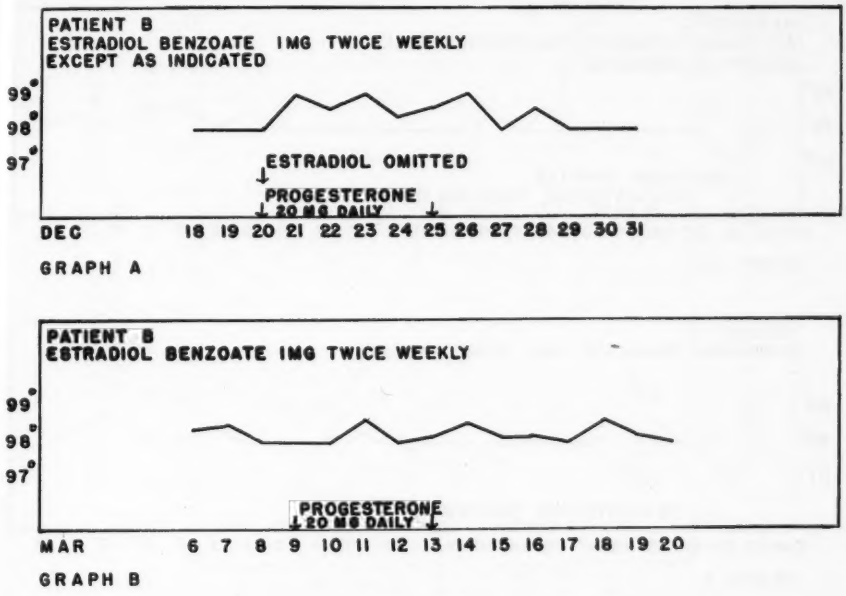

FIGURE 2

endometrium had been achieved, each patient was then given 1.0 milligram of estradiol benzoate twice a week in continuous dosage, following in exact detail the plan of maintained estrogen influence initiated with the previously described progesterone group. Upon this constantly maintained estrogen level, ten-day courses of testosterone propionate were superimposed in cyclic fashion. Dosage levels of 10, 25, 50, and 100 milligrams given daily intramuscularly were investigated. Withdrawal bleeding occurred in 100 per cent of the cases, from two to eight days following the discontinuation of the testosterone injections. Thirty-two induced menstrual cycles were observed. The patients were also followed by means of endometrial biopsy. Particularly on the higher dosage levels, considerable

luteal activity was in evidence (5). Since secretory changes in some of the biopsy specimens compared rather favorably with those seen following the administration of progesterone (in dosages of 5 or 10 milligrams given for a period of seven days) the basal temperature curves were reviewed for comparison with those obtained in patients receiving cyclic progesterone. In Figure 3, Graphs A and B show temperature charts typical of those obtained in the testosterone group with and without the omission of an estrogen injection immediately prior to the onset of the testosterone influence.

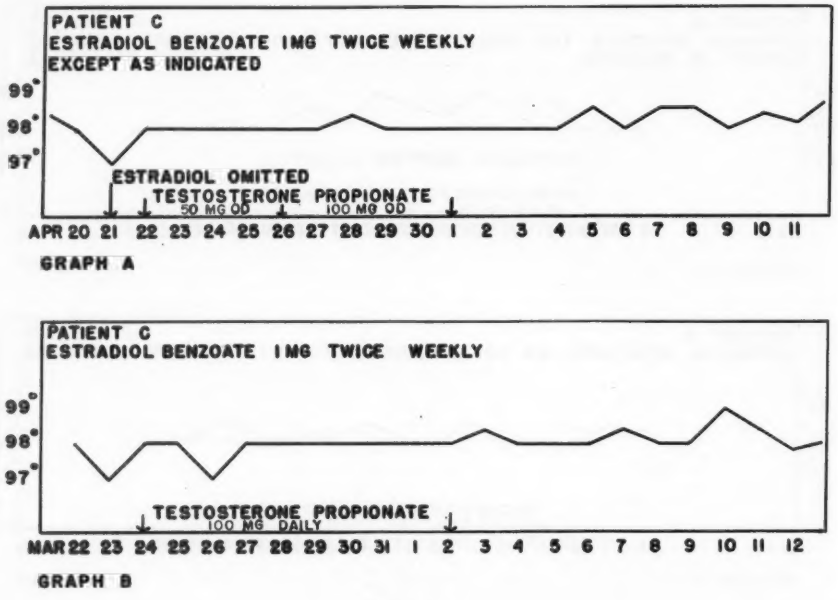
PATIENT C
ESTRADIOL BENZOATE IMG TWICE WEEKLY
EXCEPT AS INDICATED
99°
98°
97°
ESTRADIOL OMITTED
TESTOSTERONE PROPIONATE
50 MG OD
100 MG OD
APR 20 21 22 23 24 25 26 27 28 29 30 1 2 3 4 5 6 7 8 9 10 11
GRAPH A
PATIENT C
ESTRADIOL BENZOATE IMG TWICE WEEKLY
99°
98°
97°
TESTOSTERONE PROPIONATE
100 MG DAILY
MAR 22 23 24 25 26 27 28 29 30 31 1 2 3 4 5 6 7 8 9 10 11 12
GRAPH B

FIGURE 3

Since the temperature curve of the gestational period will be discussed later in conjunction with the curves obtained in the artificially induced menstrual cycles just described, Figure 4 is presented at this time for continuity. This chart, constructed in part from a table of urinary estrogen and pregnanediol values in Hamblen's "The Endocrinology of Woman" (6), correlates the urinary estrogen and pregnanediol levels during pregnancy with the typical gestational temperature curve.

DISCUSSION

The observations presented concerning the progesterone group correspond well to those made by Buxton and Atkinson (2). Their patients re-

ceived 3 to 5 milligrams of estrone sulfate or, in some instances, diethylstilbestrol daily for a period of two weeks. Following this, various amounts of progesterone ranging from 10 milligrams to 25 milligrams daily were administered for from seven to fourteen days and during this time the estro-

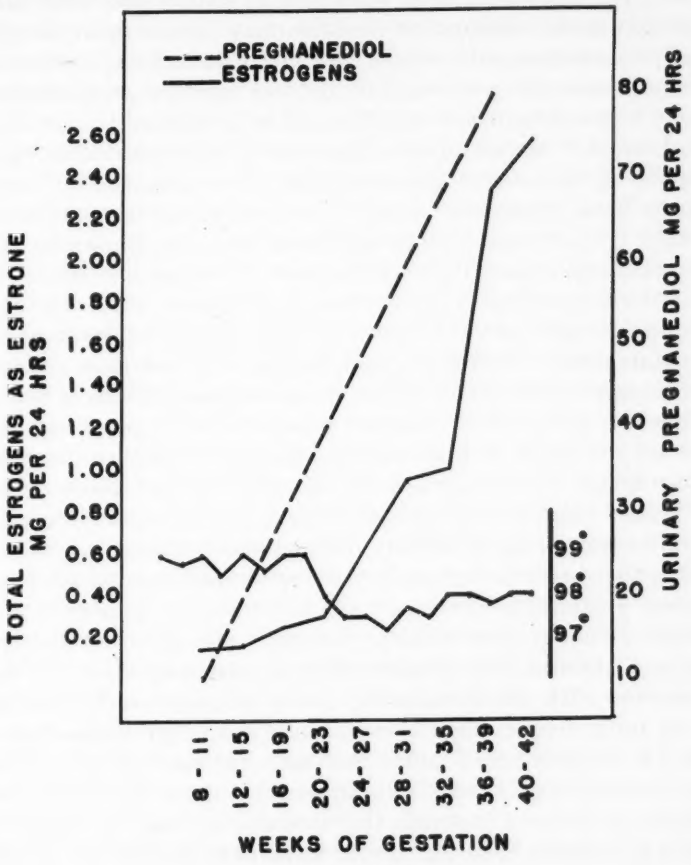

FIGURE 4

gen was either discontinued or maintained at half the original level. Typical progestational temperature rises were recorded. Davis and Fugo (3) were able to record similar rises although they continued their initial dosage of 0.3 milligram of ethinyl estradiol daily throughout the twelve-day period of progesterone administration.

It was not possible in the experiment described here to record temperature rises with a sustained estrogen level although the choice of estrogen

preparation used and the dosage differed from that of Davis and Fugo. The progesterone dosages were comparable. It would appear that at least when dosages of estrogen which probably approach physiologic levels are used in combination with adequate amounts of progesterone, the progesterone may not be the sole factor in causing a rise in temperature. Rather, the initiation of the temperature elevation may depend upon the relative levels of progesterone and estrogen at a given time. Thus, in the normal cycle may be seen the postovulatory drop in estrogen concomitant with the rise of corpus luteum activity. It could be postulated that at the time of ovulation when the estrogen is depressed to a definite critical level in relationship to the rising progesterone level, the mechanism for the elevation of the basal temperature is set in motion. At the termination of the cycle when both estrogen and progesterone levels are known to fall, the two hormones again reach their critical levels of relationship (critical ratio), allowing the temperature to return once more to preovulatory levels.

This premise also proves interesting when applied to the temperature curve of pregnancy. Farris (7), and Buxton and Atkinson (2) in their studies of temperature curves during the gestational cycle have noted that the rise which occurs with ovulation is maintained, if pregnancy ensues, until about the fourth or fifth month of gestation and then drops to preovulatory levels. In the light of our observations and those of Buxton and Atkinson which indicate progesterone as an important factor in temperature control during artificially induced menstrual cycles, it is indeed surprising that in the gestational cycle the temperature should drop at a time when progesterone levels are steadily rising. A number of theories have been advanced in an attempt to explain this apparent discrepancy. Buxton and Atkinson have postulated that a refractory state may develop in connection with the thermogenic action of progesterone or that the action of progesterone may be overcome by stronger thermodepressant factors. The observation of Caffier that the proteolytic activity of trophoblastic tissue grown in culture disappears at about the fourth to fifth months has given rise to the idea that this activity may be responsible for the "febrile reaction" during the corresponding months of pregnancy. These and numerous other theories fail to reconcile completely the events of the menstrual and of the gestational cycles in regard to temperature behavior.

The observations presented here have led to the premise that the rise and decline of the basal temperature during the menstrual cycle may depend not upon the level of progesterone alone, but rather upon the relative amounts of estrogen and progesterone present at a given time. This reasoning transposed to the thermal shift of the gestational cycle provides an explanation for the type curve seen in pregnancy (Fig. 4). In the course of

a normal pregnancy, it is known that both estrogen and progesterone values rise continuously until just prior to the onset of labor, at which time there is a sudden drop in the levels of both hormones. However, a study of Figure 4 will serve to point out that the rise of the progesterone level is extremely sharp in contrast to that of the estrogen during the first trimester of pregnancy. In fact, it is not until about the fifth month of pregnancy that the rise in estrogen parallels that of the progesterone.

It is possible that during the ovulatory period of the normal menstrual cycle in which conception occurs, estrogen and progesterone levels reach the proper ratio necessary for the initiation of the progestational temperature rise. If conception occurs, instead of the hormone levels falling at the time of the expected menstrual period, both continue to rise. The estrogen level lags, however, while the progesterone level rises sharply. This difference in the rates of rise of the levels maintains the progesterone ascendancy and does not allow the hormones to reach the "critical ratio" necessary for the depression of the temperature mechanism. Between the fourth and fifth months of gestation the estrogen level rises more sharply and parallels that of the progesterone rise. The fall of the temperature to preovulatory range at the fourth or fifth months of gestation then might be accounted for by the fact that although the absolute values of both hormones are much higher than in the menstrual cycle it is at this time that the estrogen and progesterone levels reach the "critical ratio" necessary for the inhibition of the mechanism which serves to maintain the higher temperature levels. This might be termed the "critical ratio of gestation" to set it apart from the "critical ratio of menstruation," although both ratio values may well be the same. Obviously, the testing of this concept must await more accessible methods of determining the daily levels of estrogen and progesterone during the normal menstrual cycle so that they will be available for ratio comparisons to those of the gestational cycle.

A discussion of the luteal effects of testosterone when its influence is superimposed upon an estrogen-stimulated uterus is not in the province of this paper. It has been presented in detail in a previous publication (5). It is of interest, however, that despite the appearance of a secretory type of endometrium that is obtained with testosterone exhibition, there is no significant temperature rise during the course of the testosterone injections. This is true whether or not the estrogen level is reduced by the omission of one of the bi-weekly estradiol benzoate injections (Fig. 3).

Even though the temperature rise so characteristic of the estrogen-progesterone "critical ratio" could not be produced, certain other phenomena were of interest. It will be noted in Figure 3 that the basal temperature continues as practically a straight line in both Graphs A and B during or shortly after the onset of the testosterone therapy until the withdrawal

period. This lack of what has previously been considered the normal variant of the basal temperature was consistently true in each of the 32 induced testosterone withdrawal periods that were observed. The authors are at a loss to explain the apparent suppression of the normal variant of the base line temperature, and these observations are only presented at this time as interesting phenomena. Further experience with the newer concept of testosterone withdrawal bleeding may well lead to a satisfactory explanation (5).

SUMMARY

Basal temperature observations in aged females during sex hormone replacement therapy are presented.

The results of 260 observed menstrual cycles induced by progesterone withdrawal in estrogen stimulated uteri are discussed with relation to observed basal temperature changes.

A possible correlation between the basal temperature changes of gestation and those of the menstrual cycle is suggested.

The basal temperature recordings in 32 menstrual cycles induced by testosterone withdrawal in estrogen-stimulated uteri are presented purely as present observations and without satisfactory explanation of the observed variations from normal range.

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ALTERATIONS IN SERUM IODINE FRACTIONS INDUCED BY THE ADMINISTRATION OF INORGANIC IODIDE IN MASSIVE DOSAGE*†

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IT HAS been shown that in euthyroid adults on an intake of inorganic iodide limited to that ordinarily present in the diet the serum precipitable or protein-bound iodine remains relatively constant within the range of 4.0 to 8.0 gamma per cent (1, 2). Under these circumstances the inorganic iodide in serum also remains constant at approximately one gamma per cent (3, 4). If, however, the intake of inorganic iodide is greatly increased, the concentration of this inorganic fraction rises several hundred fold, or more (4, 5). The present study defines alterations in serum protein-bound or precipitable iodine which accompany such rises during the administration of inorganic iodide in massive doses.

MATERIALS AND METHODS

The pertinent clinical data on 8 patients who received up to 3 grams of potassium iodide or up to 7 ml. of a saturated solution of the same salt daily during periods as long as six months are presented in the legends to Figures 2 through 9. The sera of these subjects were analyzed at intervals for concentrations of a) total iodine, b) protein-bound or precipitable iodine, and c) thyroxine iodine. Following suitable preparation the iodine present in each sample was measured in duplicate or triplicate by the method of Barker, with certain slight modifications which will be described. This procedure is based on the catalytic action of inorganic iodide in the arsenious acid reduction of ceric sulfate (6).

Total iodine was determined in duplicate aliquots of serum, 3.00 ml. each or less, following digestion and distillation. If the serum aliquot were less than 0.5 ml., 3 ml. of a 6 per cent glycine solution was added to provide sufficient organic material for the digestion process. This was also necessary whenever the standard aqueous solutions of

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iodide were analyzed. The digestion followed Barker's procedure, save that to insure an adequate excess of oxidizing agent a larger volume, 3.5 ml., of 60 per cent chromic acid was used with the 25 ml. of 70 per cent sulfuric acid. These reagents were mixed with the serum in a two-necked distillation flask and heated over a micro burner until the heavy fumes of sulfur trioxide appeared. The mixture was then cooled, diluted with 15 ml. of water, and reheated until the very first appearance of fumes. In the subsequent distillation process 25 ml. of water was first added. The digestion flask was then attached to a modified Chaney still and heated, following the introduction into the still head of 1.2 ml.

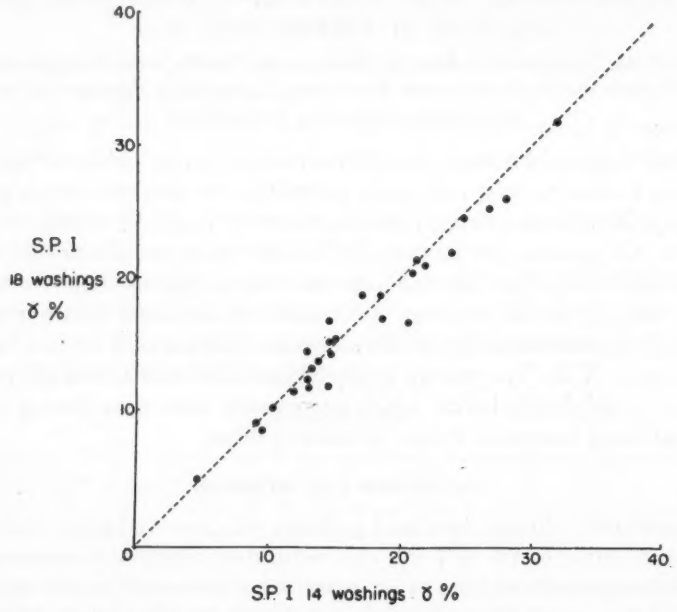

FIG. 1. In 27 samples of sera from subjects receiving massive doses of KI close agreement is apparent in the results of duplicate or triplicate analyses following 14 and 18 separate washings of precipitated sera. This clearly indicates that all of the inorganic iodide had been removed and that the remaining iodine was firmly associated with the protein.

of a 1:10 mixture of 0.1 *N* sodium arsenite and freshly prepared 0.1 *N* NaOH. When condensate began to return from the condenser, 6.0 ml. of 50 per cent phosphorous acid was added slowly to the flask. The distillation was allowed to proceed for ten minutes. The distillate was then drained into a 25 ml. volumetric flask and the still head rinsed with several 2.5 ml. aliquots of water which were in turn added to the volumetric flask. Aliquots of 5.00 ml. of distillate, 0.5 ml. of 0.15 *N* arsenious acid and of 0.033 *N* ceric sulfate were added to the colorimeter tubes. A standard curve based on known solutions, and a reagent blank consisting of a blank distillate to which a known amount of iodide had been subsequently added were included in each set of analyses. The catalytic effect of the iodide in the distillate, and in the known solutions, on the reduction of ceric sulfate by

arsenious acid was then allowed to proceed for twenty minutes at 30 ±0.1 degrees centigrade. Following this reaction period the color change in the ceric sulfate was measured in an Evelyn colorimeter, with a #420 filter and a 6 ml. aperture. In summary therefore the procedure as described differed from that employed by Barker in that a) the substitution of NaOH–Na arsenite in the trap of the still for Na_2SO_3 eliminated the need for aeration of the distillate, b) the use of the Evelyn colorimeter, a lower temperature, and longer reaction period in the final step permitted measurement of a larger number of tubes, c) the inclusion of standards and reagent blanks with each set of analyses made

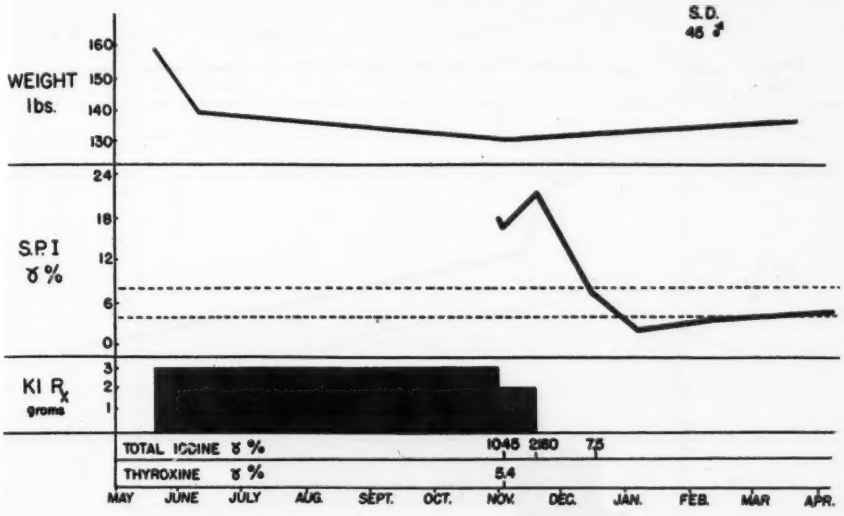

FIG. 2. KI had been started in May in this Italian barber with pulmonary fibrosis, polycythemia and congestive failure. Rest, digitalis, and P^{32} administration brought prompt relief and a diuresis, lowering the weight to 130 lbs. In the sixth month of KI therapy the SPI was found to be as high as 21.0 gamma per cent without thyrotoxicosis. Withdrawal of the KI was followed by euthyroid, hypothyroid, and ultimately again euthyroid levels of SPI as defined by the broken lines at 4 and 8γ per cent. The thyroxine concentration of 5.4γ per cent in November indicated that the SPI rise was limited entirely, or almost entirely, to the nonthyroxine fraction. The total iodine values, greatly elevated during KI therapy, returned to 7.5 following cessation of iodide intake.

possible the more precise measurement of small changes, and d) the addition of glycine in the recoveries of potassium iodide, diiodotyrosine, and thyroxine contributed no iodine and hence eliminated errors which might conceivably arise in the use of dialyzed dog serum. With these modifications, recoveries of inorganic iodide and of diiodotyrosine have been 95 to 100 per cent in the range of iodine concentrations found in serum.

In the analyses for *protein-bound iodine* the serum was first precipitated with $ZnSO_4$ –NaOH in two sets of aliquots in duplicate or triplicate and was washed by resuspension in 25 ml. portions of double distilled water. The supernatant fluid was discarded following centrifugation. In specimens obtained at a time when iodide was not being administered 6 successive washings proved sufficient to remove all inorganic iodide. Fourteen

washings were employed however for samples which contained high concentrations of inorganic iodide as a result of a high intake. The number of changes of wash water which were used in these studies may have been in excess of actual needs, but they assured complete separation of the inorganic and protein-bound fractions. This is supported by the excellent agreement obtained in the simultaneous analyses of 27 sets of aliquots washed 14 and 18 times, respectively (Fig. 1). Neither did this large number of washings remove protein-bound iodine. Serum samples washed 6 times gave the same values as duplicates washed 14 and 18 times following the in vitro addition of inorganic iodide in amounts sufficient to raise the concentration to 2000 gamma per cent (3).

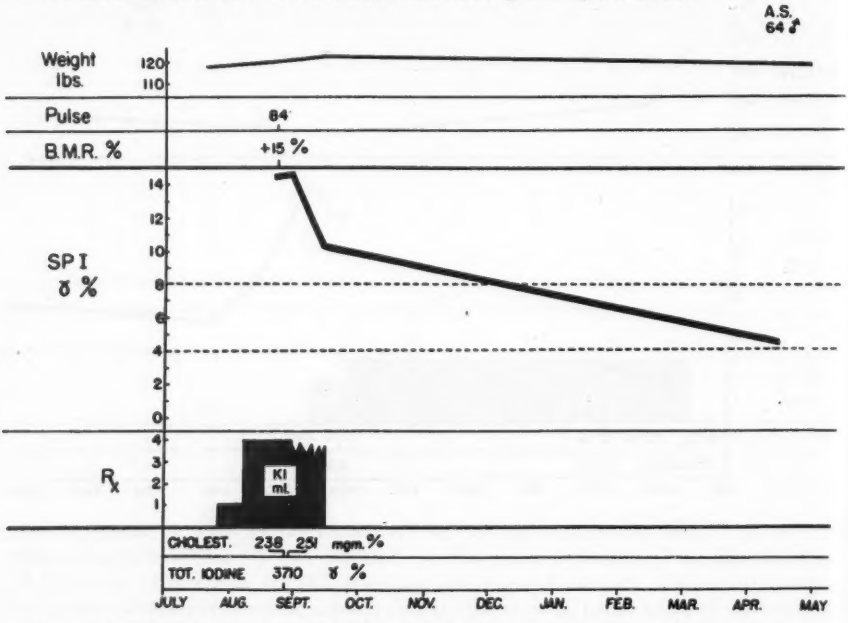

FIG. 3. KI had been started in therapy of pulmonary fibrosis and bronchiectasis. After four weeks the SPI proved to be 14.4γ per cent without hyperthyroidism; the BMR and pulse rates were not elevated and the serum cholesterol did not drop. At the time the KI was stopped, following considerable irregularity in intake as indicated by the jagged figure, the SPI was 10.3γ per cent. It subsequently returned to euthyroid levels without change in clinical status.

In the analyses for *thyroxine* the method of Taurog and Chaikoff (7, 8, 9) was used. Aliquots of 3.00 ml. of serum in triplicate were extracted with 3 successive 15 ml. portions of butanol. This procedure removed all of the thyroxine and variable amounts of the nonthyroxine iodine present in the samples of serum. The butanol extracts were in turn extracted with a mixture of 5 per cent Na_2CO_3 in 4 *N* NaOH to remove nonthyroxine iodine. A 50 ml. and then a 30 ml. aliquot of alkali was employed. In sera from subjects receiving iodide salts, 3 additional 30 ml. portions of alkali were used to insure complete extraction of the inorganic iodide. Control studies established the adequacy of these 5 extractions to remove the inorganic iodide without lowering the thyroxine content (3). The butanol

fraction was then washed into a distillation flask with 10 ml. of butanol and evaporated to dryness by vacuum distillation at less than 100°C. The iodine content of this residue, consisting of thyroxine, was subsequently determined as described above. The concentrations of thyroxine iodine, as in the case of the protein-bound iodine, were therefore measured directly. Using this method, on the average a 10 per cent loss of added thyroxine has been observed by us. This approximates in magnitude the losses reported by others (7). *Nonthyroxine protein-bound iodine* was then calculated as the difference between the protein-bound and the value for thyroxine iodine. Similarly, the level of *inor-*

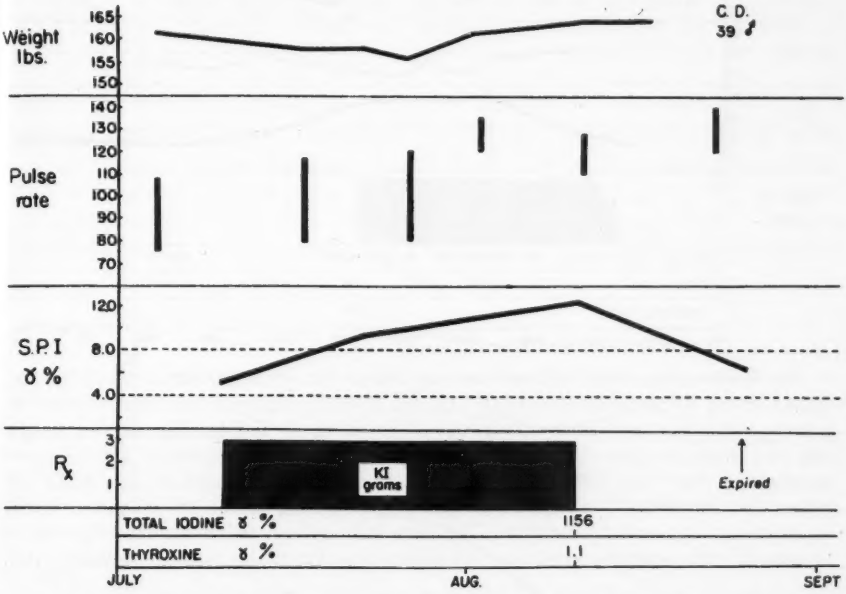

FIG. 4. The institution of KI therapy in this patient terminally ill with carcinoma of the lung raised the total iodine and the SPI; withdrawal of this medication restored normal values. The thyroxine concentration was found to be quite low, 1.1 gamma per cent, at the end of KI treatment. The fluctuations in the body weight were probably related to inanition followed by edema formation, and the tachycardia to the remittent fever associated with an extensive secondary lung abscess.

ganic iodide in any sample has been taken to be the difference between the protein-bound iodine and the total iodine concentrations as determined by the analytic procedures described earlier.

In a limited number of sera the protein-bound iodine has been partitioned between the albumin and globulin fractions of the serum proteins. In these studies the globulin precipitating techniques of Majoor and of Milne were employed (10, 11). The albumin in a 25 ml. aliquot of the filtrate was then precipitated with Somogyi precipitating reagents after modifying the concentrations of the zinc sulfate and sulfuric acid so that 1 ml. of the reagent could be used instead of 24 ml. The resulting protein precipitate was then washed and analyzed for its iodine content. The results of all analyses together with avail-

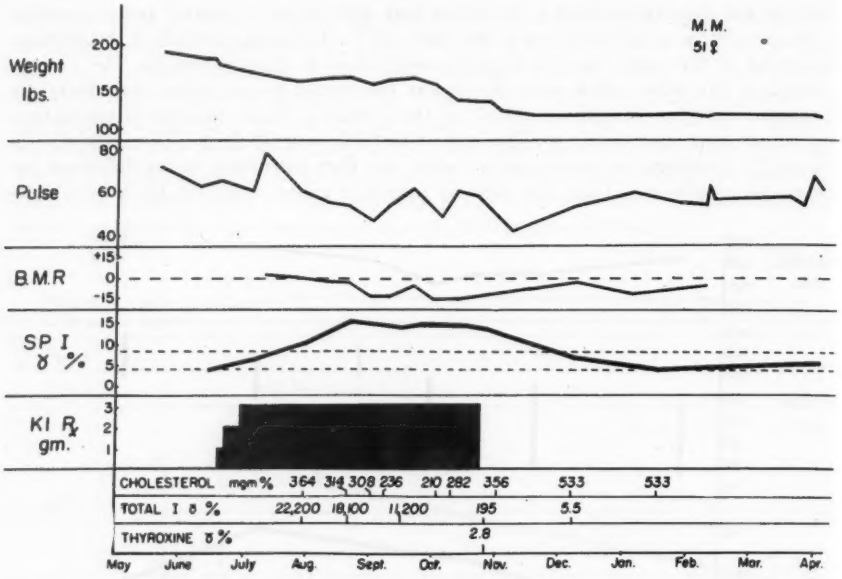

FIG. 5. Elevation of the SPI and the total iodine, but not of the serum thyroxine, occurred during KI administration in this subject ill with a nephrotic syndrome. Return to pretreatment values followed withdrawal of the inorganic iodide. The marked weight loss was related to delivery of edema fluid on a "LoNaLac" regime and not to hypermetabolism, since the patient remained in positive nitrogen balance and the BMR fell rather than increased. The pronounced bradycardia during KI therapy coincided with a supervening myocardial infarction. The serum cholesterol fell concomitantly with a remission in the nephrotic syndrome; no explanation is available for the subsequent rise.

able measurements of the body weight, pulse, basal metabolic rate and serum cholesterol are presented in Figures 2 through 9, and in Table 1.

RESULTS

A clear pattern of changes is discernible in these patients as a group, permitting certain generalizations. First, it is evident from the data that in each of the 8 patients the administration of inorganic iodide in sufficient amounts was associated with the appearance or the presence of elevated levels of total and of precipitable iodine in serum. The first of these was tremendously increased, reaching concentrations as high as 22,200 gamma per cent (Fig. 5). It is to be noted that these total iodine values during essentially uniform iodide therapy varied in magnitude from patient to patient, and even in the same patient from time to time. All were markedly increased, however, above the upper limit encountered in control subjects (3, 4). Second, the protein-bound iodine was found to be definitely elevated

TABLE 1. BINDING OF IODIDE TO SERUM PROTEINS

Patient	Date	Serum alb., (Gm. %)	Serum glob., (Gm. %)	SPI (γ %)	SPI with alb.	
					(γ %)	(% of SPI)
L.T.	9/8	3.34	3.46	21.5	18.5	86
M.M.	9/14	2.18	3.44	12.8	9.9	77
E.K.	9/3	2.69	4.30	28.0	21.4	76
A.S.	8/29	2.94	5.48	14.9	14.2	95

above the euthyroid range of 4.0 to 8.0 gamma per cent in all but the 2 myxedematous subjects (Figs. 8 and 9). The maximal levels recorded during iodide therapy in the 6 others ranged from 12.4 to 31.8 gamma per cent. These rises are therefore of a much lower order of magnitude than those observed in the total iodine. On the other hand the changes were in a sense more consistent, in that frequently the rise was progressive and ultimately

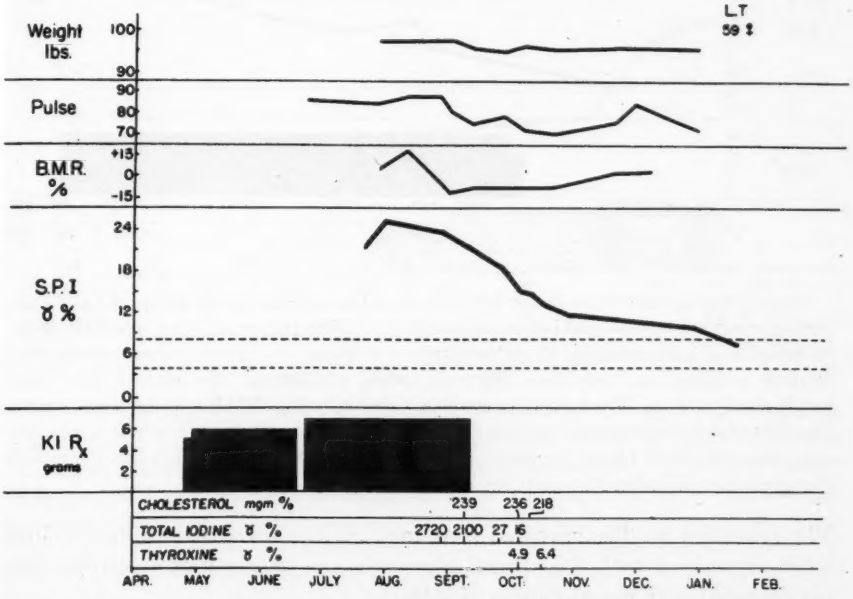

FIG. 6. Measurements of the serum iodine during iodide therapy of tertiary syphilis revealed the usual increases in the total and serum precipitable fractions; these were confirmed in repeated analyses. Withdrawal of the iodide intake was followed by a return of the SPI to the euthyroid range without change in the serum cholesterol. The thyroxine values found at a time when the SPI was high, support the pulse, body weight, BMR, and clinical data indicating that the patient did not have thyrotoxicosis.

leveled off as a plateau. This is particularly well demonstrated in patients M.M. and E.K. and is suggestively present in subjects C.D. and L.T. (Figs. 5–7). This disproportion between the increments of total and of protein-bound iodine quantitatively reflect, of course, the degree to which the circulating inorganic iodide had risen. Third, it is to be noted that the thyroxine levels were not increased during potassium iodide therapy despite the concurrent rises in total, in inorganic, and in protein-bound iodine.

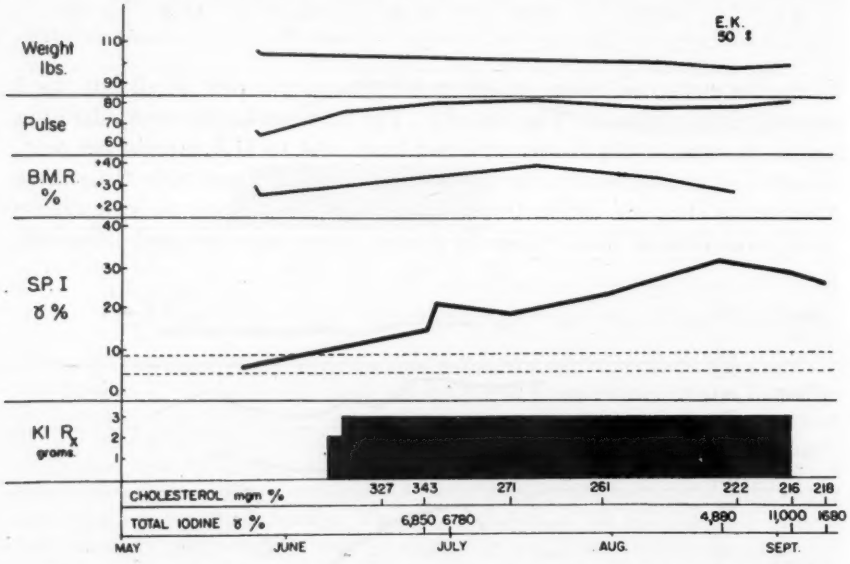

FIG. 7. A progressive rise in the SPI concentration was observed during KI administration which raised the total iodine to as high as 11,000γ per cent. There is no unequivocal proof available excluding hypermetabolic or hyperthyroid effects save that at postmortem examination, nine days following iodide withdrawal, the thyroid gland was histologically normal. The further increase in an already high BMR and the slight weight loss coincided with prolonged periods of inanition and fever during the course of a severe and ultimately fatal biliary cirrhosis. It seems reasonable also to assign the cholesterol changes to similar extra-thyroidal and extra-SPI factors.

The partition studies revealed that most of the increased protein-bound iodine remained with the albumin fraction and only minor amounts were precipitated with the globulins (Table 1).

Cessation of iodide therapy resulted in a fairly prompt return of inorganic iodide (total minus protein-bound) levels toward or to normal. Thus in subject M.M., whose total iodine during therapy had reached as high as 22,200 gamma per cent, a concentration of 195 was found five days following withdrawal of potassium iodide, decreasing ultimately to 5.5

gamma per cent. Comparable but less marked changes were observed in some of the other patients in whom total iodine values could be measured following iodide therapy. The elevations in serum precipitable or protein-bound iodine, on the other hand, appeared to persist beyond the point at which the inorganic iodide had returned to values present in healthy adults not receiving iodide supplements (Fig. 6). This observation is of some importance in the further exclusion of analytic artefact due to high inorganic

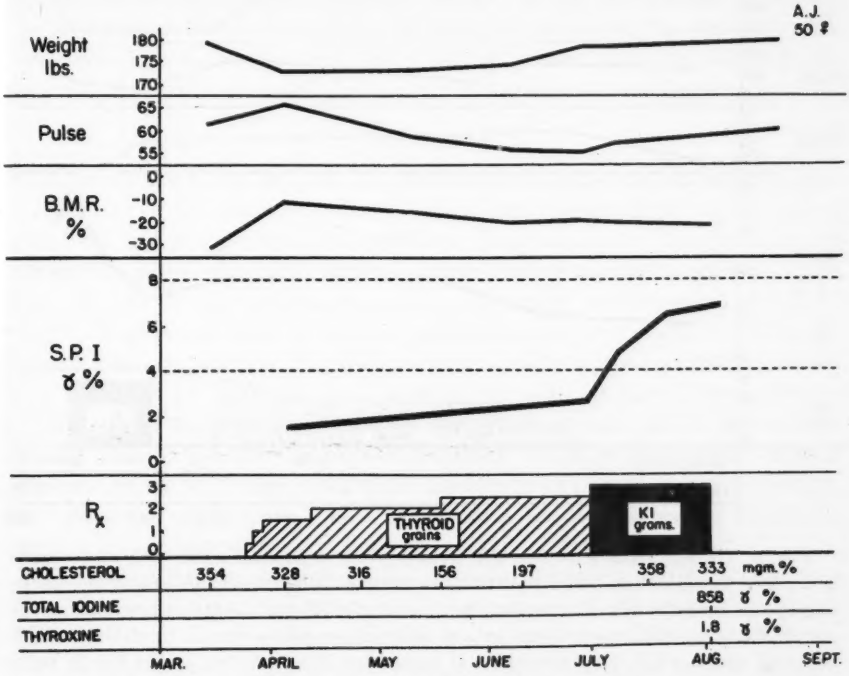

FIG. 8. The substitution of KI for thyroid in this hypothyroid female raised the total and SPI iodine values, but clinical myxedema nonetheless recurred. This is evident in the figure only in the marked recurrence of hypercholesterolemia. Study of the body weight, BMR and SPI data *prior* to KI therapy indicates, however, that thyroid replacement had been inadequate, probably because the prescribed dosage had not been maintained. There is no explanation for the slight rise in pulse rate during KI intake. It certainly cannot be attributed to any increased circulating thyroxine.

iodine as a possible explanation for the increase in the protein-bound fraction. Ultimately in all but the 2 myxedematous patients with an insufficient follow-up period and in E.K. who died before the studies could be completed, euthyroid or even lower levels or protein-bound iodine were recorded.

The clinical parallels of iodide therapy in dosage schedules of this magnitude and of the changes in serum iodide fractions which accompany it can be quickly outlined. Iodism was conspicuous by its absence. Slight papular eruptions of face or trunk which disappeared without alteration in iodide intake occurred in 3 subjects (S.G., M.M., and L.T.). Patients M.M.,

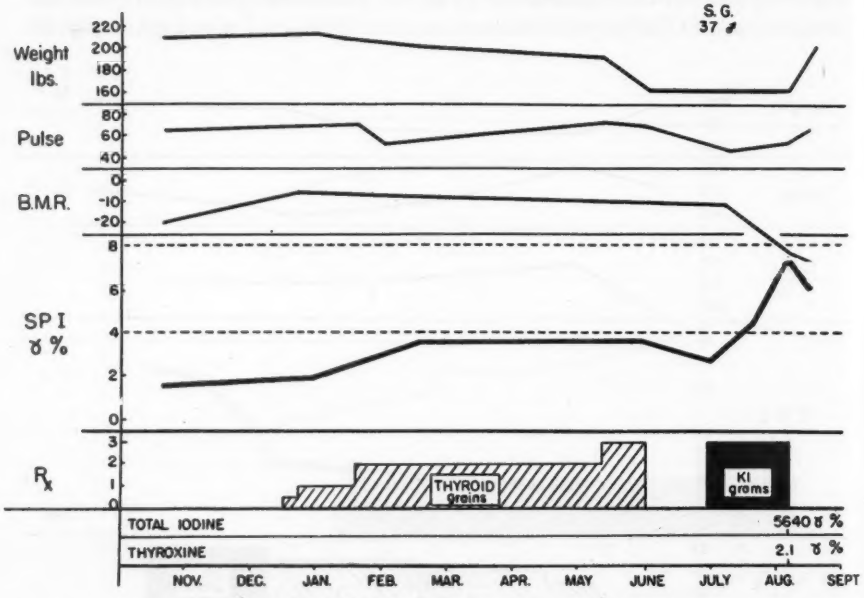

FIG. 9. Thyroid replacement in this hypothyroid, eunuchoid giant with acromegaly had raised the BMR and SPI toward or to normal. Body weight decreased markedly during diuresis following administration of desiccated thyroid, 3 grains daily. Substitution of KI for thyroid was followed by a clinical relapse with a drop in the BMR to −39 per cent, and a marked accumulation of myxedema fluid reflected in the rise in body weight, *despite an increase in the SPI*. The thyroxine analyses confirmed the clinical suspicion that the elevation in the SPI had occurred chiefly or entirely in the nonthyroxine fraction.

E.K., and S.G., all complained of a salty taste. There is no evidence that the high levels of protein-bound iodine were accompanied by hypermetabolism. In the 6 subjects on whom basal metabolic rate measurements could be made, the observed changes usually represented only fluctuations or slight decreases during KI therapy. Similarly alterations in pulse or in body weight, when present, did not point to any consistent trend and could well have been related to other clinical factors. Thus the tachycardia in E.K. and C.D. appeared terminally, concomitant with fever, long after the total and protein-bound iodine concentrations had become elevated. The

loss of body weight in M.M. and in S.D. represented the delivery of anasarca fluid and not a loss of tissue as a manifestation of hypermetabolism, since nitrogen and water balance studies in M.M. during the experimental period indicated retention of nitrogen during the diuresis (12). The decrease in body weight in E.K. accompanied marked inanition without evidences of hypermetabolism. Finally serum cholesterol analyses failed, except in 1 patient with myxedema (A. J., Fig. 9), to yield any conclusive alterations correlating with iodide therapy or iodine fraction increases. In M.M. and E.K. the interpretation is complicated, however, by the presence of a nephrotic syndrome and of biliary cirrhosis respectively, diseases known to be associated with alterations in serum lipids.

DISCUSSION

A number of facts support the validity of the observation that the protein-bound or precipitable iodine of serum can increase significantly during the daily oral administration of iodide in large amounts. The possibility of artefact is excluded by the demonstration that 14 separate washings of the precipitated serum sufficed to remove all traces of unbound inorganic iodide. This is established both by calculation of the residua which would be left following such serial dilution, as well as by actual analyses of separate aliquots of the same sera washed 14 and 18 times, respectively. Furthermore, the pattern of rise in the protein-bound iodine in contrast to the considerable fluctuations in inorganic iodide argues against simple contamination as an explanation for the increases in this fraction. Finally, the elevations of protein-bound iodide persisted long after the inorganic moiety had returned to normal limits.

The probable composition of this protein-bound iodine increment and possible mechanisms resulting in its appearance merit some discussion. The thyroxine analyses immediately exclude the possibility that the increases represent changes in this fraction. The rises would appear to have occurred, therefore, in the nonthyroxine portion of the protein-bound iodine. No data are available as to whether this indicates merely that compounds such as iodinated tyrosine ordinarily present in serum had increased in concentration or whether new substances had been formed. For practical purposes it may be considered to represent some type of firm association of iodide or iodinated compounds and serum proteins, chiefly albumin from the quantitative point of view. This could have occurred simply as the result of an in vivo iodination of protein or amino acid molecules analogous to that which can be produced in vitro (13–15). A possible though not necessarily indisputable argument against this hypothesis is the stringency of the conditions necessary for the occurrence of this reaction outside of the body. We have been unable to iodinate serum proteins

by incubation with high concentrations of inorganic iodide at body temperatures in the incubator during relatively short periods up to five days in length (3). On the other hand, it is also possible that the iodinated complex was elaborated by the thyroid gland itself. Experimental studies in rats have indicated that large doses of iodide can increase the amount of thyroxine in the gland itself (16). If this is taken to indicate that thyroxine production has increased, it is conceivable that the thyroid gland can manufacture other iodinated compounds in increased quantities. This is not unreasonable in view of the fact that it is known that the iodination of amino acids in the gland occurs in several steps (17). The finding that in the 2 patients with hypothyroidism, A.J. and S.G. (Figs. 8 and 9), the protein-bound iodine rose to 6.8 and to 7.8 gamma per cent, respectively, after a very brief interval of iodide therapy (five weeks), does not provide an answer, since residual thyroid tissue may have been present.

Irrespective of the actual mechanisms involved, the clinical significance of these findings is clear. It is obviously not possible in patients receiving large doses of iodide over extended periods of weeks or months to use the serum precipitable or protein-bound iodine as an index of thyroid function. Hence, in such subjects it may ultimately prove more wise to rely upon the level of thyroxine rather than of protein-bound or serum precipitable iodine in evaluating thyroid activity.

SUMMARY AND CONCLUSIONS

1. The administration of inorganic iodide in amounts as high as seven grams per day was associated with increased levels of the inorganic and of the protein-bound or precipitable iodine of serum.

2. These rises in protein-bound iodine appear to have occurred in the nonthyroxine moiety.

3. There was no evidence of a concomitant increase in metabolism in presumably euthyroid or in proven myxedematous subjects.

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SERUM IODINE FRACTIONS IN SUBJECTS RECEIVING POTASSIUM IODIDE IN SMALL DOSAGE*

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PREVIOUS studies from this laboratory have shown that the ingestion of an inorganic iodide in amounts as large as 3 to 7 grams per day produced a marked elevation of the total as well as the serum precipitable or protein-bound iodine (1, 2). Fractionation of the latter moiety indicated that the rise had occurred in the nonthyroxine iodine (3). Without iodide supplements, however, these various iodine fractions are known to remain relatively constant in healthy adults (4, 5). The experiments herein reported define the effects of an inorganic iodide on serum iodine fractions when administered in therapeutic amounts such as those used, for example, in hyperthyroidism.

MATERIALS AND METHODS

Five adult male subjects received 0.2 and subsequently 0.4 gram of potassium iodide daily as a saturated aqueous solution during periods totalling approximately four to seven weeks in length. Five others were given 0.6 gram daily in the form of compressed tablets for variable periods of time. All of the subjects continued their usual hospital, laboratory, or school activities in the spring and early summer months without alteration in their ordinary food habits.

The total, the protein-bound or serum precipitable, and the thyroxine iodine levels were measured at intervals, often on two consecutive days, prior to, during, and following the course of potassium iodide. The analytic procedures employed have been described in detail in an earlier publication from this laboratory (3). The method of Barker (6), slightly modified, was used to measure the iodine content of samples subjected to preliminary treatment which separated the protein-bound and the thyroxine iodine

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from the inorganic fraction. The procedure of Taurog and Chaikoff was employed in the thyroxine analyses (7, 8, 9). In the final calculations:

Inorganic iodine = total iodine minus protein-bound iodine.

Nonthyroxine protein-bound iodine = protein-bound iodine minus thyroxine.

RESULTS

Inorganic iodine. Prior to the administration of potassium iodide this fraction of the serum iodine, when measured, varied between zero and 0.7

TABLE 1. TOTAL AND INORGANIC IODINE CONCENTRATIONS IN SERUM DURING IODIDE ADMINISTRATION

Subject	Dose, (grams)	Time, (days)	Total I, (gamma %)	Inorganic I,* (gamma %)
D	0	0	5.0	0.4
	0.2–0.4	29	299	292
G	0.2–0.4	29	186	177
M	0	0	6.7	0.3
	0.2–0.4	29	247	238
P	0	0	5.8	0
	0.2–0.4	29	225	217
W	0	0	4.8	0.7
	0.2–0.4	50	227	220
B	0.6	18	826	819
Gi	0.6	17	1,065	1,060
Wi	0.6	15	869	861
R	0.6	32	1,700	1,690
H	0.6	47	648	635

* Calculated as the difference between total iodine and the protein-bound iodine on the same date as recorded in Figures 1 and 2.

gamma per cent, representing in each instance the difference between the total and the protein-bound levels (Table 1). The circulating inorganic iodide, as noted by others (10), increased markedly following the institution of iodide therapy. In the 5 subjects who ultimately took 0.4 gram per day, the inorganic fraction was found to range between 177 and 292 gamma

per cent at or about the last day of potassium iodide intake. In the 5 others receiving 0.6 gram daily the highest recorded value was 1690 gamma per cent and the lowest 635. In the limited number of observations available there is no evidence of a cumulative effect, since the subject with the most prolonged intake of iodide, H in Table 1, had the lowest total and inorganic iodide concentrations.

Protein-bound iodine. Prior to the administration of potassium iodide the serum of the various subjects was found to contain concentrations of protein-bound iodine which fell within the euthyroid range of 4.0 to 8.0 gamma per cent characteristic of healthy adults (11, 12). Analyses of sera withdrawn twenty-four hours later, when available, checked closely with the initial values (Figs. 1 and 2).

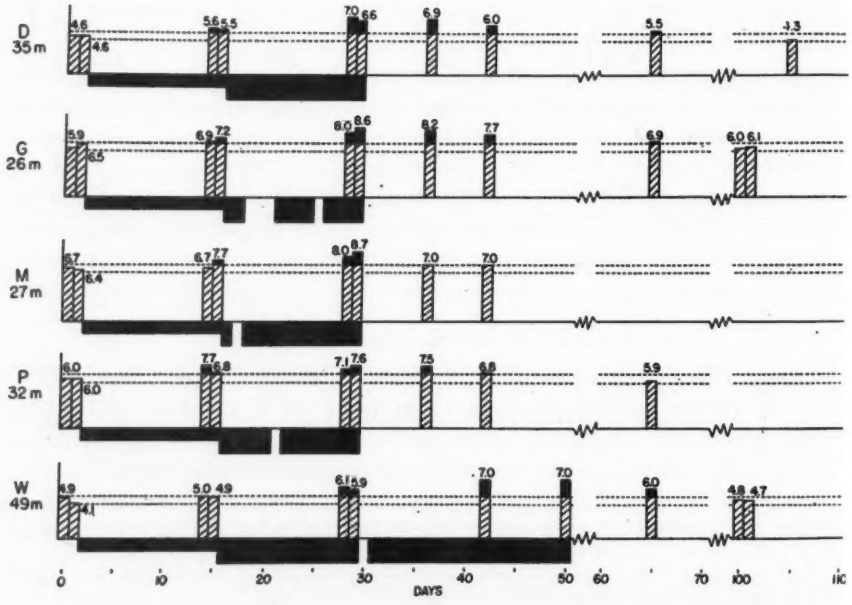

FIG. 1. Each of the vertical columns and the superimposed numbers represents the concentration of protein-bound iodine as measured on the stated day in duplicate aliquots of serum. The dotted line encompasses a range of 1.0 gamma per cent, equally distributed about the average of the pretreatment PBI values; this range exceeds the small variations in PBI usually seen in healthy adults not receiving extra inorganic iodide (4).

It is readily evident that on the last days of KI administration (0.2 gram per day for two weeks followed by 0.4 gram per day as indicated by the solid horizontal bars) the PBI had risen above pretreatment levels. These increments have been marked off in black on the columns. Subsequent to withdrawal of KI the PBI concentrations returned to the range of initial values.

In all 5 subjects who received 0.2 and subsequently 0.4 gram of potassium iodide for a total period of one month or longer the protein-bound iodine values at the end of iodide therapy were definitely higher than those obtained at the start of the experiment (Fig. 1). The increments to the pretreatment levels ranged from 1.3 to 2.2 gamma per cent. They exceed by a wide margin the analytic error of approximately 0.3 gamma per cent inherent in the method and represent changes which are unidirectional, progressive, and statistically significant.[1] In 2 of the individuals,

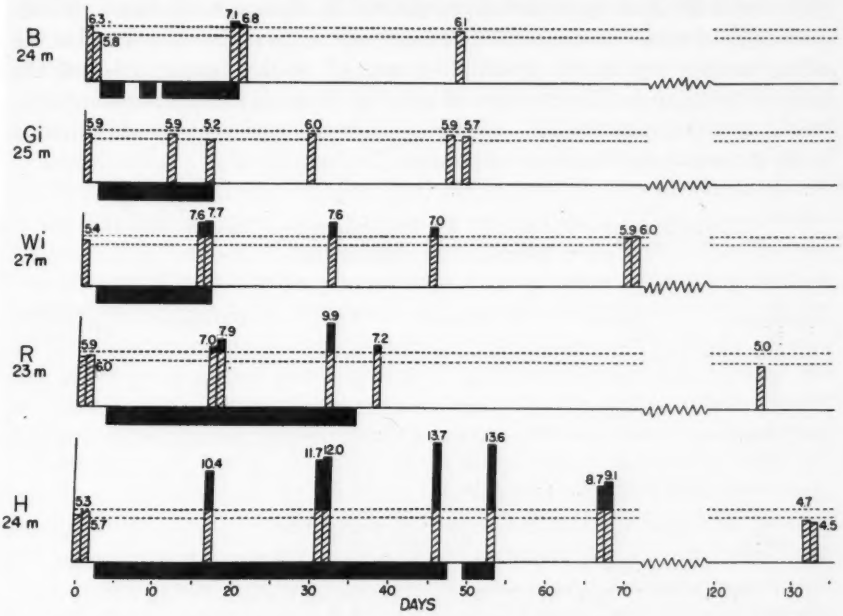

FIG. 2. Symbols as in Figure 1. The administration of 0.6 gram of inorganic iodide per day for two weeks or longer clearly raised the PBI in subjects Wi, R., and H. Pretreatment concentrations were gradually restored following the withdrawal of KI.

G. and M., concentrations of 8.6 and 8.7 gamma per cent were reached, values distinctly above the euthyroid range. Even more pronounced rises to 9.9 and 13.7 gamma per cent were observed in 2 of the 5 subjects in the 0.6 gram series (Fig. 2). They were maintained on this amount for 3.5 and 7 weeks, respectively. In the remaining 3 on this same dosage for 2 weeks a definite rise was observed only in Wi. Following the cessation of iodide

[1] The authors are indebted to Carrol S. Weil of the Mellon Institute for computation of significance of the data in Figures 1 and 2. In all but subject W., the deviations from normal mean by the "t-test" are statistically significant and "p" = <0.05; in subject W. "p" was found to be <0.10.

treatment the protein-bound iodine gradually decreased in all subjects in whom a rise had occurred. However, the elevations were still evident though less pronounced for at least several weeks later.

Thyroxine. The thyroxine data clearly indicate that the rises in protein-bound iodine which have been described occurred in the nonthyroxine fraction. From Table 2 it is uniformly apparent that thyroxine did not rise concomitantly with increases in the protein-bound iodine. There were no detectable clinical evidences of an increased metabolism in any of the subjects; serial BMR determinations in subject R. showed no increase. Whatever slight changes were recorded in the levels of thyroxine at the end of the iodide periods are in all probability related to the complexities of the analytic techniques. The absence of a rise in thyroxine in proportion to the rises in protein-bound iodine, of course, lowered the percentage of thyroxine in the protein-bound iodine component (Table 2).

TABLE 2. SERUM THYROXINE VALUES RELATED TO PROTEIN-BOUND IODINE AND IODIDE INTAKE

Subject	Dose	Days	PBI	Thyroxine	Thyroxine-PBI ratio
D	0	0	4.6	3.4†	.74
	0.2–0.4	14*	6.9	3.1	.45
G	0	0	6.2‡	4.0	.65
	0.2–0.4	14	8.3‡	3.9	.47
M	0	0	6.4	4.5	.70
	0.2–0.4	14	8.0	4.2	.53
P	0	0	6.0	4.0	.67
	0.2–0.4	14	7.1	3.2	.45
W	0	0	4.5‡	3.7	.82
	0.2–0.4	41	7.0	3.2	.46

* Ending six days earlier.

† Analysis of pooled serum obtained on two consecutive days.

‡ Average of separate analyses in duplicate on two consecutive days (*see* Fig. 1).

DISCUSSION

It is obvious that a rise in the precipitable or protein-bound iodine of serum can also be induced by the administration of small amounts of inorganic iodide. It is true that in some instances the alterations were minimal and that at times the upper limit of the euthyroid range was not ex-

ceeded. There are however several reasons for believing that the changes were not fortuitous but rather related to the intake of inorganic iodide. First, earlier studies had established that on an intake of iodide limited to that present in ordinary diets in western Pennsylvania the protein-bound iodine remains essentially constant over periods up to two months in length and perhaps even longer (4). Maximal fluctuations observed in that study were usually significantly less than one gamma per cent, and whatever changes did occur within that range were divided about equally between increases and decreases. In this study only increases were observed while the subjects were receiving iodide and these frequently exceeded one gamma per cent in magnitude. In several instances the increment was sufficiently large to place the protein-bound iodine into what has usually been considered to be the hyperthyroid range (12, 13). Technical errors related to analyses have been eliminated in these studies by obtaining two sets of observations a day apart in most of the subjects. The excellent agreement in the data which have been presented would indicate that the protein-bound iodine had actually changed. Finally, the possibility that the rises which were observed merely represented incomplete removal of the administered iodide can be dismissed on the basis of control studies. In the massive dose experiments it was conclusively demonstrated that 14 washings of the precipitated sera completely removed any inorganic iodide not bound to protein (3). In the experiments herein reported in which smaller amounts of iodide were given, a similar number of washings was employed.

Possible mechanisms whereby these alterations might have been induced can be proposed. The work of Taurog and Chaikoff with rats demonstrated that the administration of limited amounts of iodide in the diet was accompanied by an increase in the thyroxine of the thyroid gland and a rise in the serum protein-bound iodine (14). They did not measure the levels of circulating thyroxine. In our studies, assays for thyroidal thyroxine were of course not feasible, but such measurements were made in serum. The failure to observe any significant changes in circulating thyroxine or in the level of metabolism would suggest that the rise in protein-bound iodine in our studies was not related to an increased production or release of thyroxine by the thyroid gland. The possibility must be considered that the thyroid gland may have participated in the production of the rise in protein-bound iodine by elaborating nonthyroxine compounds such as diiodotyrosine. The studies of Wolff and Chaikoff indicate however that with plasma inorganic iodide levels above 20 to 35 gamma per cent, formation of organic iodine compounds in the thyroid is suppressed (15). The alternative explanation that the change represented merely an in vivo iodination of proteins or other compounds must therefore be kept in mind. The several hundredfold increase in inorganic iodide recorded in each

study indicates that the circulating inorganic iodide was certainly available for such a process.

These findings raise certain questions. First, is the pituitary capable of differentiating between this iodinated protein and the true circulating hormone of the thyroid? Our own studies are of too limited duration to afford a final answer, but the thyroxine level was not conclusively depressed in any of the subjects. It is still possible, of course, that further prolongation of the experiments might have yielded evidence of decreased thyrotropic hormone secretion, or of diminished thyroxine production. Second, does this process explain some of the paradoxes occasionally encountered in the therapy of hyperthyroidism in which the patient improves clinically, but the protein-bound iodine rises (16)? An answer to this problem should be available through the simultaneous measurement of the circulating thyroxine level and of the protein-bound iodine in hyperthyroid patients receiving therapeutic iodide solutions.

SUMMARY AND CONCLUSIONS

Increases in the daily intake of inorganic iodide, 0.2 to 0.6 gram for two or more weeks, were frequently accompanied by a rise in the protein-bound iodine of serum. At times the changes in this fraction were of sufficient magnitude to exceed euthyroid values, but the circulating thyroxine level remained essentially unchanged.

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PANHYPOPITUITARISM: CASE REPORT CORRELATING CLINICAL AND PATHOLOGIC FINDINGS

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INTRODUCTION

CORRELATION of physiologic abnormalities and anatomic changes in panhypopituitarism is difficult. Although many cases of this disease have been reported, there have been few in which currently available methods of evaluating endocrine function were accompanied by autopsy findings. This report describes fairly extensive physiologic studies in a female with panhypopituitarism and the subsequent autopsy findings.

CASE REPORT

First admission: J. S. (Evans Memorial Hospital #320,431), a 46-year-old Italian female entered on July 31, 1946, in semi-stupor. The history as obtained from a relative revealed that since extraction of an abscessed tooth three months previously, she had been bedridden and incontinent of urine. A productive cough and a fever developed one day prior to admission.

The patient was married at the age of 21 and divorced at 36. She had never been pregnant. Menarche was at 11; periods had been irregular for several years and had ceased, as far as could be determined, one year prior to admission. She had been listless, apathetic, easily fatigued, anorexic, and had lost some weight during this year. There was no history suggesting attacks of hypoglycemia.

Physical examination: The temperature was 101°, pulse 84, and blood pressure 120/80 mm. Hg. She was a well nourished, dehydrated white female who was unresponsive, incoherent, and unable to sit up in bed. Examination of the chest revealed slight dullness at the left base with a few moist rales and a pleural friction rub. There were old, healed decubitus ulcer scars on the heels and buttocks, and contractures of the left hamstring muscles.

Laboratory examinations: The urine specific gravity was 1.005; and the pH 5.0, with no albumin, sugar, bile or abnormal sediment. The blood Hinton test gave negative results. Blood hemoglobin was 10.5 grams per 100 cc., hematocrit 31 per cent, erythrocytes 3.54 million per cu. mm., leukocytes 15,300 to 6,200 per cu. mm. The fasting blood sugar was 83, and serum nonprotein nitrogen 30 mg. per 100 cc. On lumbar puncture the fluid was clear, under normal pressure, contained no cells, gave a negative reaction

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to the Pandy test, contained 45 mg. per 100 cc. of sugar, and 105 mEq. per liter of chloride; cultures did not show any growth. The chest roentgenogram showed diffuse mottled density throughout the entire right lung field and a portion of the left upper lung field. The electrocardiogram showed left axis deviation, shifting pacemaker and periods of auricular standstill and ventricular escape, and periods of complete cardiac standstill for as long as two seconds.

The temperature subsided after five days of penicillin therapy; there was clearing of the density in the left chest, and the patient was discharged on August 10, 1946, with a diagnosis of bronchopneumonia, and arteriosclerotic heart disease with left ventricular enlargement and shifting pacemaker.

Second admission: On September 17, 1948, she was readmitted with the chief complaints of weakness, fatigue, and cough of two years' duration. In the 2-year interval between admissions, she ate poorly and had remained in bed for several months prior to admission because of constant fatigue.

The temperature was 98°, pulse 56, and blood pressure 152/95 mm.Hg. She was apathetic, listless, but well nourished and appeared older than the stated age. The skin was dry and scaling with numerous brown macular spots. The hair of the head was receding; axillae were devoid of hair: and pubic hair was scant. (Fig. 1). There was lenticular opacity in the right eye. The thyroid was not palpable. The left border of cardiac dullness was 8 cm. to the left of the midsternal line, the heart sounds were faint and obscured by rales in the lungs, a Grade I apical systolic murmur was heard, and the rhythm was regular except for runs of extrasystoles. The liver was palpable three fingerbreadths below the right costal margin. On pelvic examination a small introitus admitted one finger with some discomfort to the patient; no masses were palpated in the adnexae.

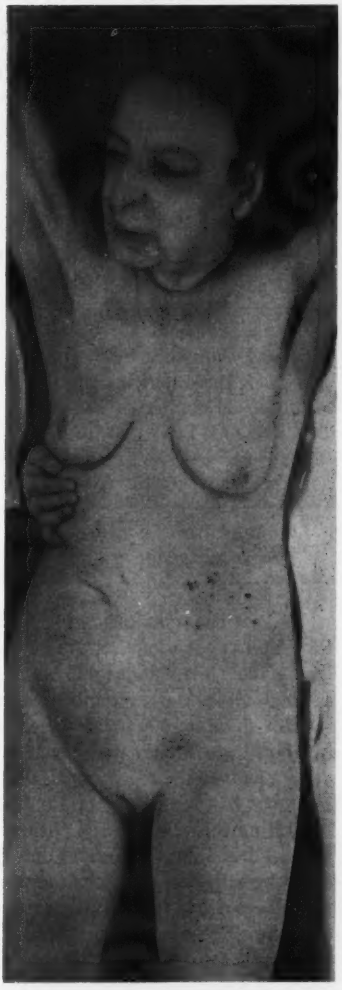

FIG. 1. Patient J. S.

The urine maximal specific gravity was 1.026, pH 7.5, and albumin varied from 0 to +; the sediment contained leukocytes, erythrocytes, rare granular casts, and gram negative bacilli. The blood hemoglobin was 11.5 grams per 100 cc., hematocrit 39 per cent, erythrocytes 4.48 million per cu. mm., and the total leukocytes and differential were normal. The fasting blood sugar was 90 mg. per 100 cc. The serum protein was 6.06 grams per 100 cc., with an albumin of 2.71 and globulin of 3.35. Serum cholesterol was 198, calcium 7.8 and phosphorus 2.9 mg. per 100 cc., alkaline phos-

phatase 3.2 Bodansky units per 100 cc. Serum chloride was 83, and carbon dioxide content 25 mEq. per liter. The basal metabolic rate was −20 per cent. Radioactive iodine uptake measured 0.0 per cent before, and 7.0 per cent after administration of 30 mg. of thyroid-stimulating hormone.[1] The urinary excretion of 17-ketosteroids was 0.4 mg., and of follicle-stimulating hormone, less than 3 mouse units per twenty-four hours. During a Kepler-Power water test, the night volume of urine was 350 cc.; next morning the patient voided 120 cc. during the first hour of the test and 200 cc. during the next four. Actual hourly excretion rates are unknown because the patient was unable to void at these intervals. X-ray examinations of the skull showed no abnormality of the sella turcica. Further laboratory examinations are presented in Tables 1 and 2, and Figures 7, 8, and 9.

TABLE 1. RESPONSES OF CIRCULATING EOSINOPHILS
Patient J. S.

Substance given	Initial eosinophil count	Eosinophil count at end of four hours	Per cent drop
.2 mg. Adrenalin	266	200	25
1.5 mg. Adrenalin	488	133	73
60 cc. Adrenal extract (3,000 dog units)	622	311	50
25 mg. ACTH	333	133	60
Normal control			
.2 mg. Adrenalin	88	0	100
1.5 mg. Adrenalin	355	22	94

The patient remained lethargic, incontinent of urine, and ate poorly. On the twenty-third and twenty-fourth hospital days, 10 mg. of desoxycorticosterone acetate in propylene glycol[2] intravenously, and on the following successive days 60, 50 and 50 cc. of adrenal cortical extract intramuscularly were given (Table 2). Thereafter, 2 grams of sodium chloride, 300 grams of carbohydrate and 5 mg. of desoxycorticosterone acetate in oil intramuscularly were given daily; and 2.5 mg. of testosterone in oil was given every other day. On the thirty-ninth day, 15 mg. of desiccated thyroid daily was started and increased to 45 mg. daily on the forty-fourth day.

During this regimen the patient became more alert and cooperative and was able to walk. Early in the course of desoxycorticosterone therapy, because of pretibial edema and wheezing, the drug was temporarily omitted. During this time, there was never any significant rise in the blood pressure which varied from 112/70 to 140/80 mm. Hg.

Following subcutaneous implantation of three 75-mg. pellets of desoxycorticosterone acetate and three 75-mg. pellets of testosterone, she was discharged on November 1, 1948, on a high carbohydrate diet and 45 mg. of desiccated thyroid daily. The diagnoses were: panhypopituitarism and arteriosclerotic heart disease.

[1] We are grateful to Dr. E. B. Astwood for making these measurements.

[2] We are grateful to Dr. Edward Henderson of the Schering Corporation for supplying us with this material, and for the testosterone and DOCA pellets used later in this same patient.

TABLE 2. SERUM ELECTROLYTE CONCENTRATIONS, SALT INTAKE AND HORMONE THERAPY

Date	Serum			Therapy	
	Na	K	Cl	Na*	Hormone
	mEq./L.	mEq./L.	mEq./L.	mEq./24 hrs.	
9/21	124	5.8	89.8		
9/30	132	5.0	87.2		
10/7	115	4.3			
10/8				435	
10/9	115	4.2	74.1	348	10 mg. DOCA in propylene glycol i.v.
10/10	117	3.6		174	10 mg. DOCA in propylene glycol i.v.
10/11	112	3.9	76.6		60 cc. Eschatin i.v.
10/12	113	3.8	79.1		50 cc. Eschatin i.v.
10/13	119	3.8			50 cc. Eschatin i.v.
10/14	120	3.8	83	134	10 mg. DOCA in oil i.m.
10/15				134	10 mg. DOCA in oil i.m.
10/16				134	10 mg. DOCA in oil i.m.**
10/17	130	4.0	92.4	103	
10/18				36	**
10/19				36	
10/20	132	4.5	93.4	36	5 mg. DOCA in oil i.m.**
10/21				36	5 mg. DOCA in oil i.m.
10/22				36	5 mg. DOCA in oil i.m.**
10/23				36	5 mg. DOCA in oil i.m.
10/24				36	5 mg. DOCA in oil i.m.
10/25				36	5 mg. DOCA in oil i.m.**
10/26	137	3.9	98.2	36	5 mg. DOCA in oil i.m.

* As NaCl.

** 2.5 mg. testosterone in oil i.m., in addition.

Third admission: On January 26, 1949, she again entered because of increasing weakness and drowsiness. Her physical status and laboratory examinations were essentially unchanged, except that hair was noted on the cheeks and upper lip, and she was much more lethargic and unresponsive. It was believed that she had not taken the desiccated thyroid with any regularity. She was treated with thyroid, and streptomycin for the urinary tract infection, but did not improve greatly and was discharged on February 13, 1949.

Her last hospital entry was to the Boston City Hospital in May, 1949, because of increasing jaundice for three weeks. The liver was palpated four fingerbreadths below the right costal margin and was hard and irregular. The icterus index was 100, and there was bile in the urine. She became progressively worse with persistent jaundice and died four days after entry.

AUTOPSY

Gross examination

(B.C.H.—A-49-368) eleven hours post mortem. The body was that of a normally developed, thin, but not cachectic female of Italian descent, 150 centimeters in length and 110 pounds in weight. The skin was intensely jaundiced. The hair was black, thin and receded back to a line over the vertex. There was a bald spot over the occipital region. The axillae were devoid of hair and there was a scattered, sparse growth of hair on the cheeks, lips, chin, and in the pubic region. The breasts were atrophic and on section no glandular tissue was visible.

The peritoneal cavity contained 1,500 cc. of clear yellow fluid and the omentum and mesentery of the transverse colon were adherent to a hard mass in the region of the gallbladder.

The heart, lungs, kidneys, and spleen were not remarkable.

There was a carcinoma of the gallbladder which had extended into the liver and head of the pancreas, and had metastasized to one regional lymph node. Small stones were present in the gallbladder and common bile duct.

There was bilateral atrophy of the convolutions of the frontal lobes of the brain.

Endocrine organs

The dura of the dorsum sellae was incomplete and did not cover the aperture. The stalk of the pituitary appeared normal in size and was attached posteriorly to pituitary tissue in the floor of the fossa. No pituitary tissue could be seen on initial inspection. Section, however, revealed on the floor of the fossa, flattened anterior and posterior lobe tissue, which measured 1 mm. in thickness and occupied 25 to 30 per cent of the volume of the fossa. The hypothalamus was not remarkable.

The thyroid weighed 13.5 grams, was firm and symmetrically divided into two lobes, and on section revealed the usual light brown cut surface.

The adrenals were small and friable. The combined weight after fixation was 8.1 grams. The cortices were greatly thinned and measured 1 millimeter in thickness. The medullae were bilaterally replaced by a small amount of fluid blood.

The ovaries measured $0.8 \times 0.5 \times 0.3$ cm. and were completely fibrosed. There were no visible follicles or corpora lutea in either ovary. The uterus measured $5 \times 2 \times 1$ centimeters and had a thin atrophic endometrium. The tubes were not remarkable.

Four parathyroid glands were found. Three measured approximately $4 \times 2 \times 3$ millimeters; one measured 2 millimeters in diameter. They were very soft and rusty brown.

Microscopic examination

Fixatives—10 per cent neutral formalin and Zenker's acetic acid. Stain—phloxine methylene blue.

The gallbladder, liver and head of the pancreas were invaded and replaced by dense collagenous connective tissue, in which were embedded numerous cords, clusters, and acini of anaplastic, pleomorphic tumor cells. A small amount of this tissue was present in one para-aortic lymph node.

The heart, lungs, spleen, and kidneys were normal.

Endocrine organs

Pituitary. Six serial 2-millimeter slices were made of the entire pituitary and a section cut from each slice. The anterior lobe tissue was flattened and appeared compressed (Fig. 2). The cords and acini of epithelial cells were aligned with their long axes parallel to the floor of the sella. Just above the pituitary was a cystic space lined by loose con-

nective tissue. Arachnoidal fibroblasts, several layers thick, were present at some points on the cyst lining, as well as an occasional psammoma body.

FIG. 2. Photomicrograph of pituitary and pituitary fossa. The anterior lobe is flattened into a thin strip of tissue lying on the floor of the fossa. Above it is the cystic space lined by fibrous tissue. At the bottom lies the posterior lobe. Phloxine-methylene blue. ×20.

In some areas, dense collagenous tissue had replaced anterior pituitary cells, but these were few in number and small in size.

Results of cell counts of three different areas of this pituitary were:

	I	II	III
	600 cells	503 cells	457 cells
Acidophils	26%	25%	15%
Basophils	8%	1%	19%
Chromophobes	66%	74%	66%

The acidophils were very small, not much larger than the chromophobes (Fig. 3), and none had the abundant cytoplasm seen in acidophils of normal pituitaries (Fig. 4). Many had pyknotic nuclei. The basophils were larger but not as large as normal cells. The posterior lobe was subnormal in size, but cytology was normal.

Thyroid. Most of the acini were small, lined by flattened or cuboidal cells and contained an eosinophilic colloid, in some areas granular. A moderate number contained basophilic colloid. Connective tissue was present in increased amount between the acini.

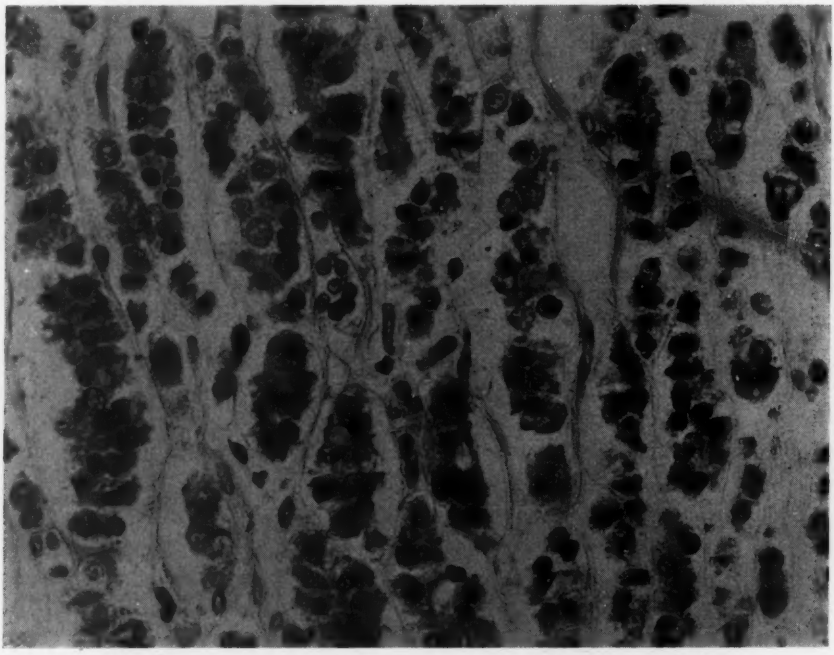

FIG. 3. Photomicrograph of anterior lobe showing the parallel cord-like arrangement of cells. The cells with pyknotic nuclei and scant cytoplasm are acidophils. Except for 2 basophils in the center, the remainder of the cells are chromophobes. Phloxine-methylene blue ×800.

Adrenals. The cortices were narrowed and appeared essentially alike. A glomerular zone was present and was made up of clusters of small cells with regular round nuclei and a small amount of granular acidophilic cytoplasm. The fascicular zone showed the greatest proportionate narrowing of the three zones. The fascicles of cells were short and showed little tendency toward the usual alignment (Fig. 5). In a very few areas these cells had a foamy, vacuolated cytoplasm, the remainder a granular acidophilic cytoplasm (Fig. 6). The reticular zone was completely absent in many places, but in some regions a few cells containing lipofuchsin pigment were present. In other areas there were small isolated nodules of pigmented cells, some of which extended out to the capsule. Medullary tissue was scant and largely replaced by red blood cells and precipitated protein material.

Ovaries. No follicles, ova, or corpora lutea were present. The ovarian stroma itself had largely disappeared and was replaced by collagenous connective tissue. A few corpora albicantia were seen.

Breast. The breast tissue consisted entirely of collagenous connective tissue and a few dilated ducts without acini.

Parathyroids. Chief cells predominated. Very few "Wasserhelle" cells and very little fat was present. There were several large clusters of large oxyphil cells.

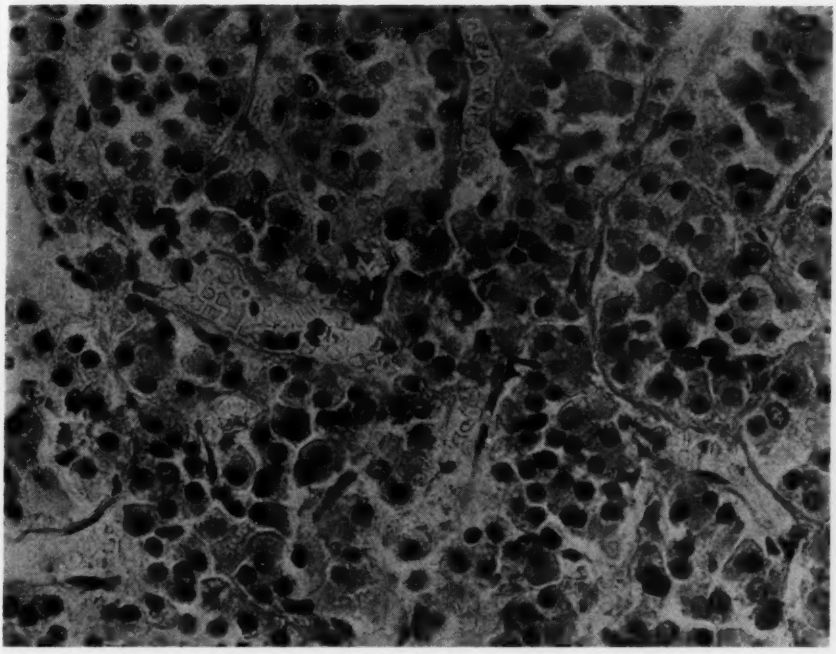

FIG. 4. Photomicrograph of normal pituitary for comparison with Figure 3. Most of these cells are acidophils with abundant cytoplasm. Phloxine-methylene blue. ×800.

Diagnosis: Panhypopituitarism with thyroid, adrenal and ovarian atrophy, adenocarcinoma of the gallbladder, with invasion of the liver, head of the pancreas and metastases to regional nodes, cholelithiasis.

DISCUSSION

The precise cause of the partial destruction of the pituitary is difficult to determine. Two possibilities exist: first, the defect in the dura over the pituitary allowed intracranial pressure to compress pituitary tissue; second, the cavity occupying most of the fossa was a cyst of the meninges which caused atrophy by steady accumulation of fluid with expansion.

There can be little question that the physiologic abnormalities in this patient were adequately related qualitatively to the anatomic changes in the endocrine glands, even though an explanation for the actual cause of the atrophy of the pituitary was not apparent. Quantitative comparison of the degree of atrophy of the pituitary and of the other endocrine glands with functional evidences of abnormality, is not uniform.

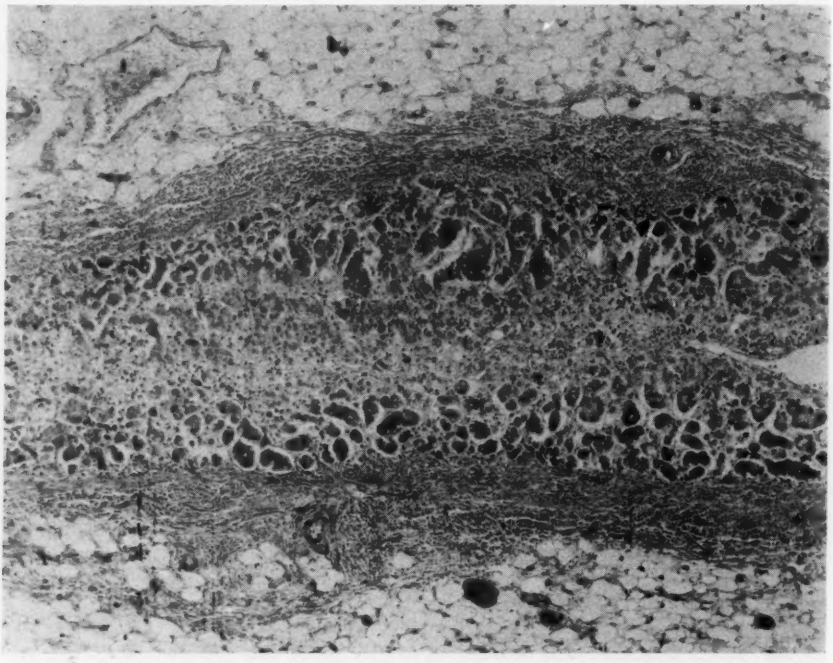

FIG. 5. Photomicrograph of adrenal showing narrowing of all zones, but particularly the fasciculata. There is no medullary tissue in this field. Phloxine-methylene blue. ×100.

Estimation of the amount of pituitary tissue remaining in panhypopituitarism is clearly important because the clinical manifestations frequently vary widely. For example, cachexia may be an outstanding feature, or entirely absent (1, 2). Evidence of hypoadrenalcorticism of the degree seen in Addison's disease is usually lacking, but is sometimes present (3, 4, 5). Not infrequently the clinical state resembles myxedema more closely than any other (1).

The above mentioned clinical variations are not surprising in view of the differences in the gross and microscopic findings in the pituitary, and in the glands controlled by it, which have been reported in human autopsy material (1, 4, and 6–12).

In the case reported here, it was estimated that 25 to 30 per cent of the anterior pituitary remained. The only hormone of the pituitary per se which was assayed, the follicle-stimulating hormone in the urine, was excreted in quantities less than 3 mouse units per 24 hours. The inference is that there was almost total destruction of that portion of the gland which produces gonadotropic hormones, presumably of the basophils (13). The

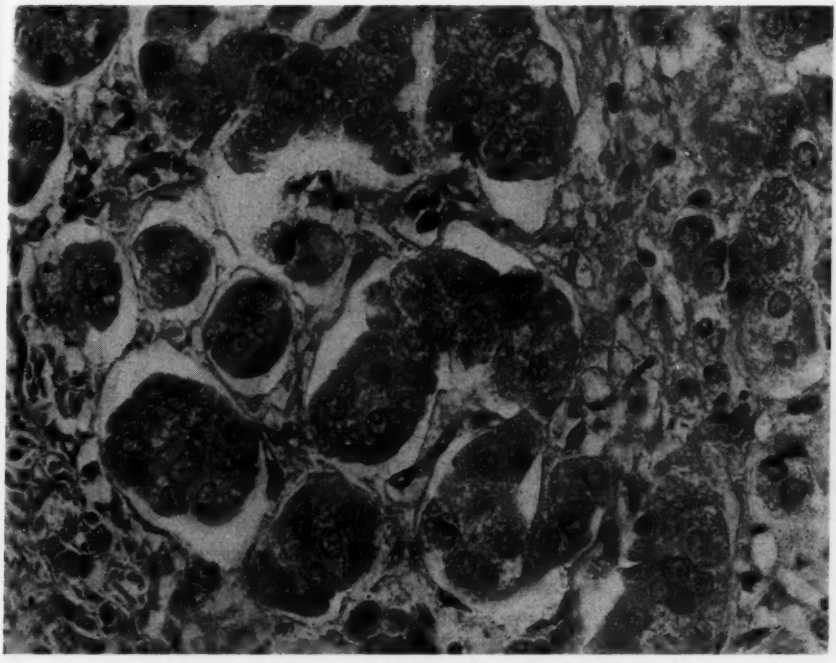

FIG. 6. High power view of adrenal cortex showing granular cytoplasm of cells of the zona fasciculata. Phloxine-methylene blue. ×800.

ovaries showed complete atrophy histologically, and were the only endocrine organs under control of the pituitary in which no functioning tissue remained. One might argue that this finding represented changes resulting from a physiologic menopause, but in the authors' opinion the degree of atrophy far exceeded that usually seen following a normal menopause.

Thyroid function was considerably depressed clinically, and the basal metabolic rate was minus 20 per cent. The ability of the gland to respond to pituitary stimulation was conclusively demonstrated by the absence of uptake of radioactive iodine prior to, followed by definite uptake subsequent to, administration of thyroid-stimulating hormone. Anatomically

the gland was only moderately reduced in size, and microscopically showed small follicles and flattened epithelium.

The characteristic insulin sensitivity and hypoglycemia unresponsiveness furnish good evidence of absence of those hormones of the pituitary which contribute to control of carbohydrate metabolism, although part of the carbohydrate abnormalities are clearly attributable to decreased formation of adrenocortical hormone (Fig. 7).

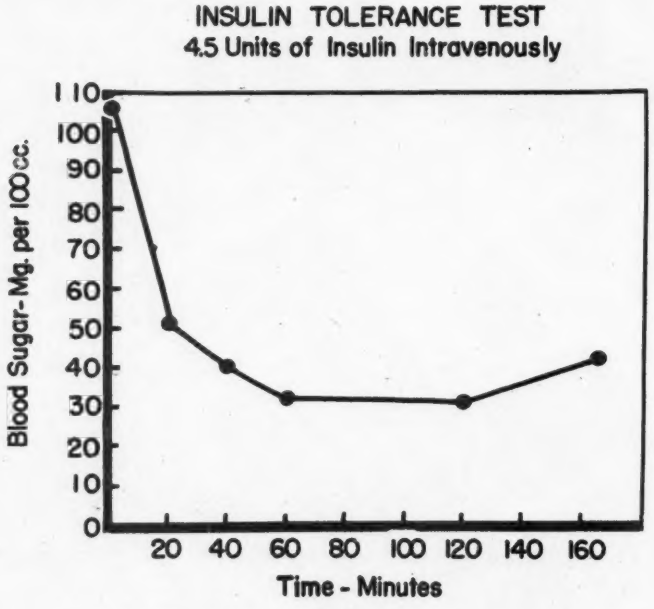

FIG. 7. Insulin sensitivity and hypoglycemia unresponsiveness during insulin tolerance test.

Anatomically the adrenals were markedly reduced in size, but a small amount of tissue from all three zones remained, with the greatest reduction in the size of the reticularis and fasciculata (Figs. 5 and 6). The studies of adrenal cortical function are of especial interest in view of these findings. The responses of circulating eosinophils were the expected ones. Thus, administration of adrenocorticotropic hormone (ACTH) resulted in a decrease in the number of circulating eosinophils, indicating that the adrenal cortex was capable of mobilizing steroids. The decrease was similar to that which followed administration of adrenal cortical extract. The responses to epinephrine further demonstrated that the pituitary, under the stress of a large dose, was able to produce some ACTH; but with the dose effective in

the normal subject, only a moderate reduction in eosinophils resulted (Fig. 8). The response to ACTH is apparently not always as clear-cut as it appeared to be here. Thus, Forsham *et al.* observed a drop of 4 to 45 per cent in 5 cases of hypopituitarism and a rise of 24 per cent in 1 case (14). The low urinary 17-ketosteroid excretion and the positive water test (in absence of renal impairment) further confirm the evidences for hypoadrenalism. The responses to insulin, as mentioned previously, were also unques-

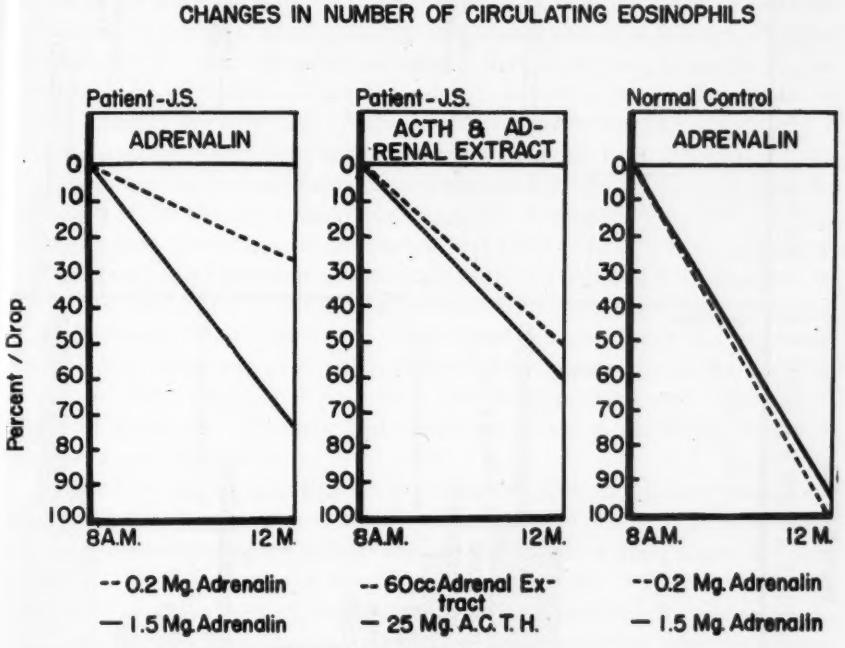

FIG. 8. *See* text for discussion.

tionably partially a result of hypofunction of the cortex (14, 15). The abnormalities of electrolyte metabolism further indicate deficiency of those adrenal steroids controlling salt and water metabolism. There were hyponatremia, hypochloremia, and slight hyperpotassemia which were corrected by administration of DOCA and sodium chloride (Fig. 9). Prior to administration of DOCA the patient was excreting in the urine quantities of sodium and chloride which were excessive considering the presence of hyponatremia and hypochloremia; in addition, during most of this period, she was eating very little, and hence her electrolyte intake was negligible. This inability to conserve sodium and chloride by the kidneys was promptly reversed by intravenous DOCA (even in the presence of an augmented salt intake), and was relatively unaffected by adrenal cortical extract. An

expected retention by the kidneys of potassium before, and an increase in urinary excretion after DOCA was not demonstrated; indeed urinary potassium excretion diminished after administration of the hormone.

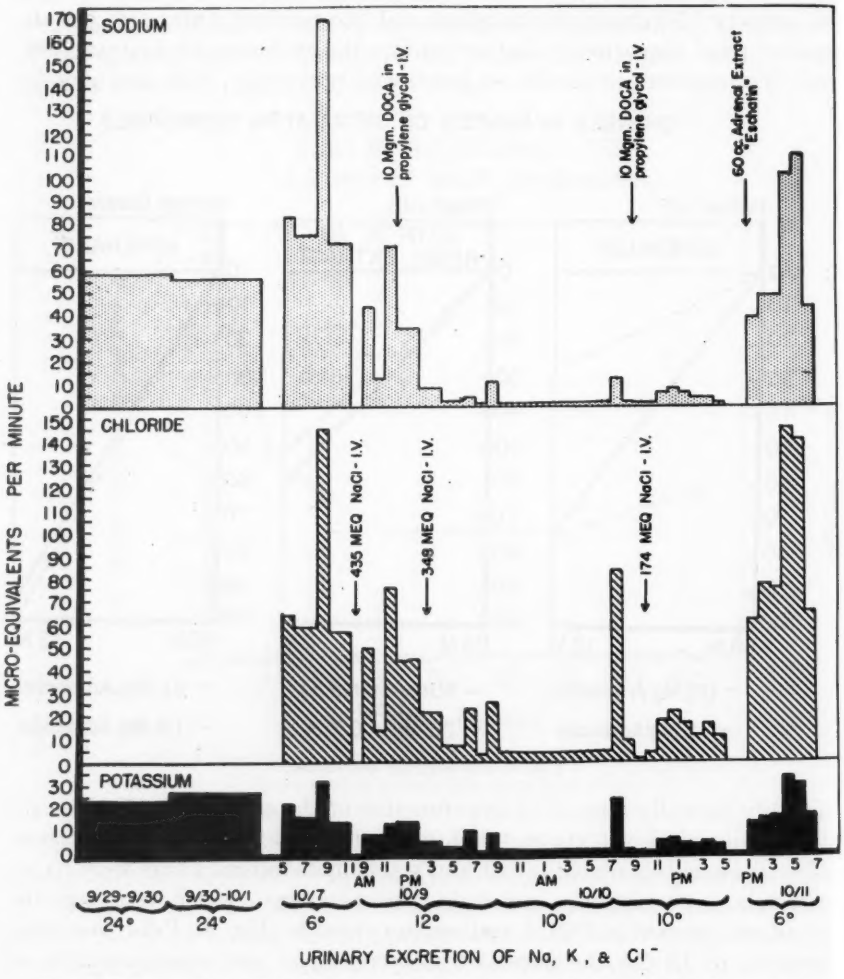

FIG. 9. Urinary electrolytes before and during treatment. *See* text for discussion.

Similar abnormalities of electrolyte metabolism have been reported previously in hypopituitarism. In 6 of 7 patients studied, Stephens demonstrated a fall in serum chloride and high urinary chloride excretion after three days of salt deprivation; 4 of these exhibited symptoms characteristic

of salt depletion (5). Five of 6 patients studied by Williams had episodes suggesting "adrenal crises" and associated hyponatremia and hypotension (16). "Addisonian crisis" and a serum total base of 134 mEq. per liter was reported by Fraser in one case following administration of thyroid (3).

The disturbances of salt and water metabolism in our case and in those just mentioned is of considerable interest. In general, most patients with hypopituitarism do not show as much disturbance in salt and water metabolism as patients with Addison's disease having comparable hypoadrenalism, by criteria not involving salt and water metabolism. Bergner and Deane (17) have presented evidence that in the rat, although the secretion of 11-oxysteroids from the adrenal cortex is under the control of the pituitary, the secretion of the desoxycorticosterone-like substances is not. In man, however, there is contradictory evidence from different sources on this point. In the long-term experiments of Forsham *et al.*, following the injection of ACTH there was definite sodium retention. This was taken as evidence that the trophic hormone had stimulated the secretion of desoxycorticosterone-like substances from the adrenal cortex. An alternative explanation was that the secretion of certain androgens might have produced this effect (14). The evidence in man would seem to favor the view that ACTH does stimulate production of desoxycorticosterone-like substances.

Hence, from functional and anatomic studies there was evidence of multiple glandular deficiency, but with some function remaining in all the glands except the ovaries.

In addition to the quantitative reduction in pituitary tissue, there were slight but definite alterations in the cytology of this gland. The proportions of the three cell types fell within the normal range as determined by Rasmussen (18). However, the chromophobe count (66 to 74 per cent) was increased over the normal average figure of 49.6 per cent for the adult female, and the acidophil count (15 to 26 per cent) was lower than the normal average figure of 43.4 per cent. The marked variation in the basophil count merely indicates the usual tendency for these cells to be grouped in certain areas of the pituitary. The most obvious cellular differences were the pyknotic nuclei and the small size of the acidophils.[3]

In the few reported cases in which counts were done on the pituitary tissue, similar, but more pronounced, changes were noted: Gallavan and Steegmann in 2 cases found 97 and 98 per cent chromophobes, 1.5 and 1.6 per cent acidophils, and .3 and .9 per cent basophils (9); Bratton and Field found a great majority of chromophobes with very few eosinophils and basophils in one case (7); and Lang found the pituitary consisted largely of

[3] It should be noted that figures for the proportion of cell types in this gland are not strictly comparable to those of Rasmussen since he used Mallory's anilin blue stain and the stain in this case was phloxine-methylene blue.

chromophobes with almost complete absence of basophils in another case (4).

An explanation for cytologic alterations in the residual pituitary tissue is not apparent. It is possible that they represent effects secondary to depressed function of the thyroid, gonads and adrenals, originally resulting from partial destruction of the pituitary. In pituitaries from castrated human individuals an increased number of basophils, large chromophobes, and inconstant changes in the acidophils, but usually a decrease in number and size, have been reported (19). Thyroidectomy was followed by a decrease in the size of acidophils with loss of specific granulation and a moderate increase in the number, size, and degree of vacuolization of basophils (20). The pituitary in Addison's disease showed variable reduction in the number of basophils, and a great reduction in the number and size of acidophils, many of which had pyknotic nuclei (21).

These studies suggest that the effect on the pituitary of ablation of the gonads, thyroid, or adrenals is somewhat similar, in that in each instance a decrease in the number and size of the acidophils, accompanied by a change in the nuclear structure of these cells, tended to occur. The pituitary of the present patient showed these same changes; little or no alteration was demonstrated in the basophils.

Thus, although the initial process in this case was undoubtedly the destruction of a major portion of the pituitary, it also seems likely that the secondary atrophy of the thyroid, gonads and adrenals in turn had an effect on the cytology of the pituitary.

SUMMARY

1. The clinical, physiologic, and pathologic findings in a female with panhypopituitarism are presented.

2. In general, the changes in the pituitary, thyroid, adrenals, and gonads correlated well with previous functional evaluation of these glands.

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Endocrine Review

A HYPERGLYCEMIC FACTOR EXTRACTED FROM THE PANCREAS*†

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THE metabolic processes of the body are governed by compensating checks and balances which are frequently interdependent, some accelerating, some inhibiting specific processes. Among the most extensively studied of these activities is that of the factors affecting carbohydrate metabolism. Insulin has, of course, been long known to be of prime importance to this process, but investigations have suggested that another substance obtained from the pancreas may also play a role in sugar metabolism. This substance has been found to produce hyperglycemia and increase the rate of hepatic glycogenolysis.

There is at present no direct proof that the pancreatic hyperglycemic factor is functional under physiologic conditions. Results of cross-circulation experiments by Weinstein, Smith and Foa (1) have suggested that this may be true, but further observations are deemed necessary. Certain other evidence, both clinical and experimental, strongly supports this possibility and shall be briefly discussed at this time.

CLINICAL OBSERVATIONS

Patients surviving total pancreatectomy require relatively small doses of insulin to control their hyperglycemia and glycosuria; 6 of 7 patients (2–6) surviving more than two months required 40 units or less daily, and the seventh required 30 to 80 units a day. It has been remarked repeatedly that the diabetic state following total pancreatectomy is not as severe as anticipated and is characterized by a marked sensitivity to insulin. This sensitivity has been attributed to the poor nutritional state of these patients (7). Two patients, previously diabetic, underwent total pancrea-

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tectomy (7, 8) and the insulin requirement either remained unchanged or decreased, despite the removal of the entire gland. In these cases all means available were utilized to overcome the poor nutritional state of the patients; but if these means were not completely successful, malnutrition may have accounted for the relatively low insulin requirements. On the other hand, deficiency or lack of a pancreatic factor which normally elevates blood sugar may also have played a part.

Although no classification of diabetes is entirely satisfactory (9) there is a certain group of patients who require relatively large doses of insulin for control and do not commonly develop acidosis, whereas other diabetic patients are sensitive to insulin and constantly hover between diabetic coma and insulin shock. These latter, as will be noted subsequently, resemble in this respect the pancreatectomized animal; and the former, resemble in some respects, certain "alloxan diabetic" animals. Many patients with diabetes require doses of insulin much in excess of the 30 to 40 units required by the human after pancreatectomy. In some instances the hyperglycemic rebound (10) following hypoglycemia may play a role but other cases are not explainable on these grounds. That the secretion of the pituitary or adrenal or other endocrine glands may account for certain of these differences is well established. The thought has been expressed, however, that a pancreatic factor such as will be discussed here may account for some of the discrepancies observed.

A review of the literature has revealed the occurrence of tumors believed to originate from islet tissue, in patients with diabetes. Warren (11) noted that 1 of 20 patients having adenoma of the islets suffered from diabetes. Of 3 of the cases studied by Lopez-Kruger and Dockerty (12) the authors stated ". . . interestingly enough . . . the islet cell tumors developed in spite of the existence of clinical diabetes mellitus." Smith and Seibel (13) speaking of one of their cases remarked, "This tumor is unlike the others . . . in that it gives neither clinical nor anatomical evidence of functional activity. In addition this is the only case in which a definite although mild diabetes was present. . . ." In other cases either no adequate studies were made, or the existence of diabetes was not mentioned. Further investigation with special staining technics and extraction procedures should be employed in future cases of this kind.

Direct evidence of alpha cell function is lacking and a few studies for alpha cell granules have been made. A case of adrenal insufficiency with superimposed diabetes has been reported (14) in which degranulation of both alpha and beta cells was found. Knowlton and Kritzler suggest: "It is conceivable that the total absence of granules in the pancreatic islets in the patient here described might indicate the lack of the normal anti-insulin of this region, and in part account for the sensitivity to exogenous

insulin." The authors point out, however, that in other cases similarly studied alpha cell granules were easily seen.

It has frequently been observed (15–18) that diabetes may occur with extensive pancreatic disease and that an occasional patient will require an amount of insulin in excess of the usual requirement noted in patients following pancreatectomy; a possible explanation is an imbalance produced by the disease process between the hyperglycemic and hypoglycemic functions of the pancreatic islets.

It was suggested by Gyorgi (19) that certain cases of Van Gierke's disease, which is characterized by low blood sugar, insulin sensitivity and the excessive storage of glycogen may be due to a deficiency of the hyperglycemic or a similar factor. Recent observations by Paschkis (20) have suggested that this may well be true in some but not all cases of Van Gierke's disease. Another interesting possibility is that the hypoglycemia so frequently observed in infants may be produced by a relative impairment of alpha cell function in early life. We have seen no reports of special staining for alpha cells having been performed on pancreatic tissue removed from infants with hypoglycemia.

EXPERIMENTAL DIABETES

Certain discrepancies have been noted in various forms of experimental diabetes which also bear on this subject. Young (21) reported on the production of diabetes in the dog by the injection of an anterior pituitary extract. He noted that the diabetes was characterized by marked hyperglycemia with relative insulin resistance. He also noted that an animal in this state would survive long periods of time even if insulin was withheld. However, when the pancreas was removed from such an animal, the blood sugar level fell, the insulin requirement was decreased and the animal was very sensitive to insulin. Such an animal survived only a short time without insulin. Somewhat similar observations were made by Dragstedt *et al.* (22) on dogs rendered diabetic by subtotal pancreatectomy. These animals were resistant to insulin and marked hyperglycemia developed, whereas after total pancreatectomy they were quite sensitive to insulin and small doses were sufficient to control the diabetic state.

Dragstedt (22) and Houssay (23) noted that in some animals, after administration of alloxan, more marked hyperglycemia developed than was seen after pancreatectomy. Of particular interest is the report of Thorogood and Zimmermann (24) on alloxan diabetic animals. These workers performed experiments on such animals similar to the experiments reported by Young (21) in anterior pituitary diabetes. They observed the marked hyperglycemia and high insulin requirements after alloxan and further noted little change after ligation of the pancreatic ducts. They also

noted the relatively slight ketonuria in alloxan-treated animals when insulin was withheld. They observed the decreased insulin requirements and increased sensitivity to insulin after pancreatectomy and further observed that on withholding insulin, marked ketonuria developed in the pancreatectomized animal. These authors postulated a secretion, possibly from the alpha cells, which raises blood sugar and possibly affects ketosis. Candela (25, 26) and his co-workers amplified these observations and corroborated the findings of Thorogood and Zimmermann. In addition, the Spanish investigators suggest that the activity described is under parasympathetic control.

This group of clinical and experimental observations suggests that a substance or factor produced by the pancreas may elevate blood sugar and that, possibly, this or another factor may affect other metabolic processes preventing or decreasing ketosis under certain conditions.

PANCREATIC EXTRACTS

Jensen (27) calls attention to many observations made during the decade 1920 to 1930 and later, concerning a contaminant of insulin which produced hyperglycemia. We shall not attempt a complete review of this literature, but it is of interest that in some protocols published by MacLeod (28) in 1922 an initial hyperglycemic activity was evident in the early preparations of Banting and Best. Murlin (29, 30, 31) and his co-workers observed that certain extracts of the pancreas as well as of other tissues caused hyperglycemia, and Burger (32, 33) reported on numerous observations of this activity. Of particular interest are the observations of de Jongh (34) who believed that the "anti-insulin" was adsorbed by insulin; this belief was based on the action-concentration curves of insulin preparations. Geiling and deLawder (35) reviewed the experiments performed in Abel's laboratories and found that with the successive steps in the purification of insulin the "inactive residues" contained a hyperglycemic substance. They found that the final crystalline insulin produced by Abel was free of this factor. Spicer, Kazal, Wolfe, and Barnes (36) have recently reported on efforts to purify the hyperglycemic factors which they have found to be present in many of the "inactive residues."

Interest in this "contaminant" was aroused recently by the observations of Bouckaert and De Duve (37) who found that a brand of insulin manufactured by NOVO[1] failed to produce effects ascribed to insulin by certain American investigators (38). Sutherland and Cori (39), and Sutherland and De Duve (40) have studied a factor extracted from the pancreas and

[1] NOVO insulin is a preparation of crystalline zinc insulin manufactured by the NOVO Terapeutisk Laboratorium, Denmark.

prepared from all brands of American insulins, but not from NOVO insulin, which produced hyperglycemia and increased the rate of glycogenolysis in liver slices. They found that this substance is nondiffusable, is not destroyed by heating to 100°C. for five minutes, and can withstand treatment by fairly strong acid and alkali; it is present in extracts of gastric mucosa and to a lesser extent in duodenal mucosa. It may be significant that cells with characteristics of alpha cells are present in these area. The hyperglycemic substance, in the dog, was extractable in higher concentrations from the tail of the pancreas where there is more islet tissue than from the head; it was also found in the pancreas after duct ligation, administration of alloxan, and in the fetal calf pancreas before the acinar tissue becomes functional. These studies on the glycogenolytic activity of the hyperglycemic factor suggest that this material is probably obtained from the alpha cells and further that it has certain metabolic potentialities quite different from insulin. Heard and his co-workers (41) remarked on a factor obtained from the pancreas which produced hyperglycemia; he felt that it probably was produced by the alpha cell. The extraction procedures used were described in detail by Stewart (42). Olsen and Klein (43) have noted that after the intravenous injection of insulin a transient hyperglycemia is regularly produced by various doses of American insulin but not by NOVO insulin.

DeWaele (44) extracted beef pancreas with fat solvents and obtained a contra-insulin. He suggests that an excess of contra-insulin may play a role in the etiology of diabetes in addition to the lack of insulin, or that an imbalance of these may be responsible.

Collip (45) and Macallum (46) have described certain crude pancreatic extracts which provoked hyperglycemia lasting for prolonged periods of time, and exerted profound effects on carbohydrate metabolism. The activities of these extracts differ from those of the material under discussion here, both in the latent period and length of action. They are presumably different in nature from the hyperglycemic-glycogenolytic factor under discussion.

These studies further confirm the fact that it is possible to extract from the pancreas a factor which elevates blood sugar. This factor is extremely difficult to remove from insulin and is present in significant amounts in all American insulins. Sutherland, Cori, Haynes and Olsen (47) have extensively studied the characteristics of the hyperglycemic-glycogenolytic substance. It is protein in nature, has an electrophoretic mobility slightly slower than insulin and can be prepared free of insulin. The factor prepared from gastric mucosa appears to be identical with that from the pancreas.

Studies have been reported by Zimmermann and Donovan (48) on the

effect of this factor in ketonuria and acidosis. However, they found no clear cut evidence for such an effect. We, too (49), have attempted to prove the factor effective in preventing death after pancreatectomy; but our results, although suggestive of such action, may have been due to small amounts of insulin remaining in our extracts and preparations and are, therefore, not conclusive. However, further studies are at present being carried out concerning the possibility of other metabolic effects of this substance.

METHODS

Extracts were prepared by Dr. S. A. Komarov by the method described by Best *et al.* (50). Tissues were obtained in the fresh state and were im-

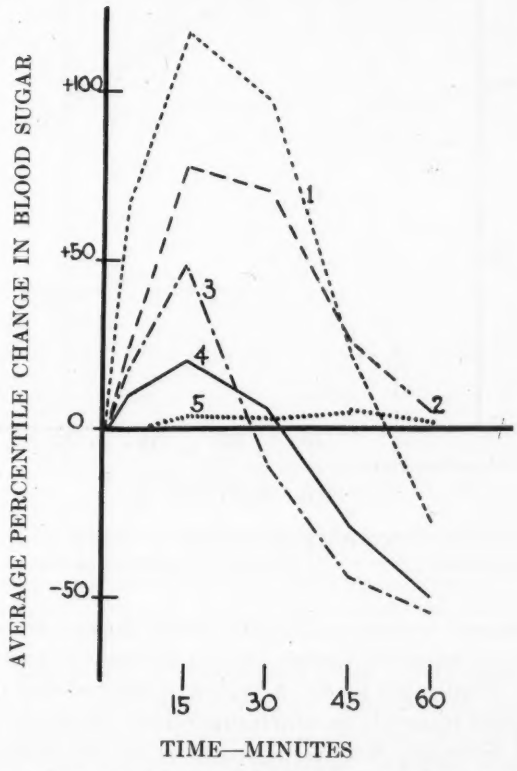

FIG. 1. Average blood sugar changes in 10-kilogram dogs following the intravenous injection of extracts prepared from 6.5 to 7.0 grams of tissue: 1. Fetal calf pancreas (– – – – –). 2. Pancreas from dogs previously treated with alloxan (— — —). 3. Dog pancreas following duct ligation (—·—·—). 4. Normal dog pancreas (———). 5. Parotid gland of dog and cattle (·····).

mediately frozen or processed. Six and one half to seven grams of tissue was used for each extraction.

Insulin preparations were inactivated by heat and alkali after the method described by Sutherland (39).

Experiments to be described were carried out on dogs weighing between 8 and 11 kilograms after anesthesia was induced by sodium pentobarbital.

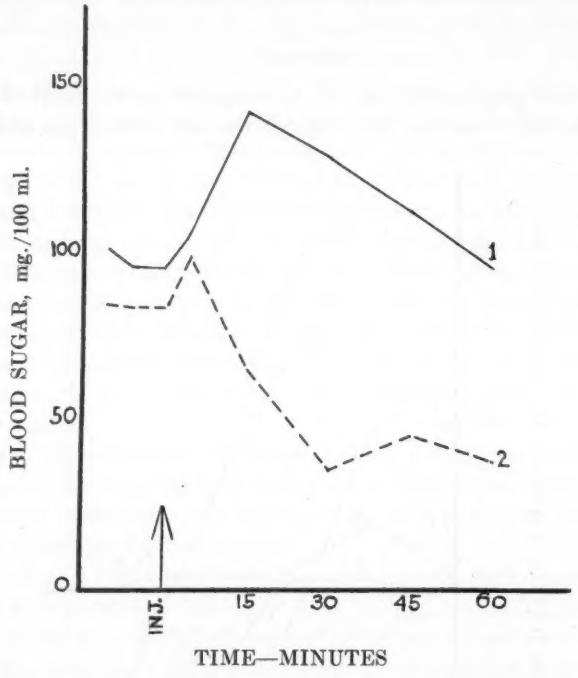

FIG. 2. Blood sugar changes following intravenous injection of: 1) 10 units of inactivated insulin (———). 2) 10 units of unaltered insulin (— — —).

Control experiments without anesthetic agents showed no significant difference from those reported. Larson (51) found that the barbiturates have little effect on blood sugar levels. A vein was exposed and blood samples taken at repreated intervals on which sugar determinations were made by the method of Folin and Wu (52) as modified for the photoelectric colorimeter.

RESULTS

1. As illustrated in Figure 1, intravenous injection of extracts of parotid gland obtained from either cattle or dogs failed to affect blood sugar levels

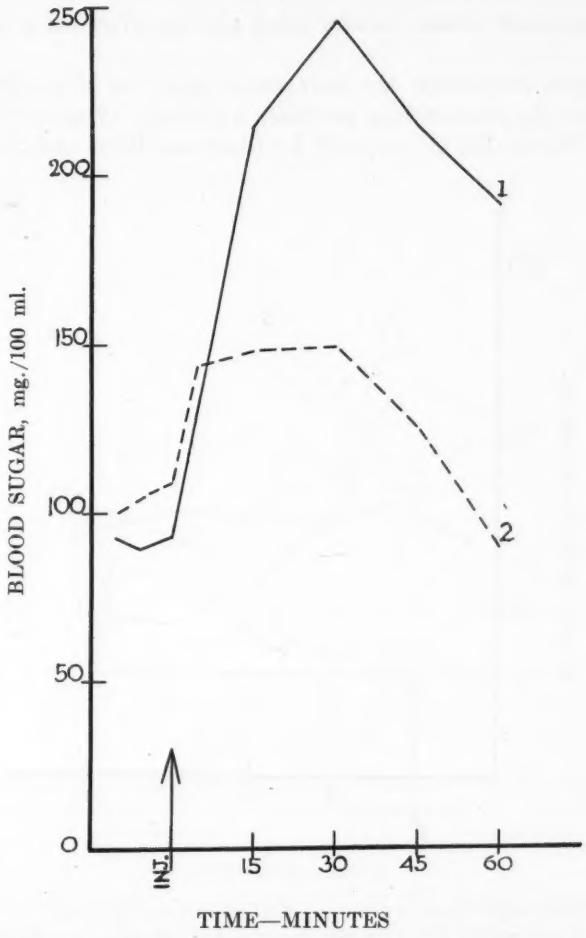

FIG. 3. Blood sugar changes following intravenous injections of: 1) 240 units of inactivated insulin (———). 2) 240 units of unmodified insulin (— — —).

significantly. Similar extracts prepared from normal dog pancreas produced first a hyperglycemia, followed by significant hypoglycemia. Comparable preparations from fibrocystic dog pancreas following duct ligation, and from fetal calf (4–5 months) pancreas, produced marked hyperglycemia followed by a fall in blood sugar. However, when such extracts were made from the pancreas of dogs previously treated with alloxan, a marked hyperglycemia without secondary hypoglycemia was produced. These experiments add significance to the findings of Sutherland and De Duve

(40) who obtained similar results using glycogenolysis as a measure of activity.

2. As stated previously, the intravenous injection of various doses of American insulin preparations provokes a primary hyperglycemia as described by Mason (53) and recently by Olsen and Klein (43). Insulin that

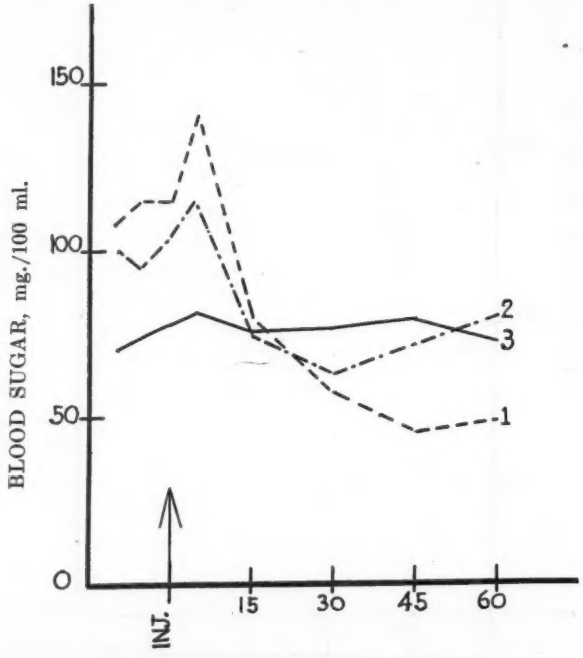

FIG. 4. Blood sugar changes following the intravenous injection of NOVO insulin. 1) 240 units of unmodified NOVO insulin (— — —). 2) 240 units of partially inactivated NOVO insulin (— · — · —). 3) 240 units of inactivated NOVO insulin (———).

has been inactivated with heat and alkali causes a more marked and more prolonged hyperglycemia, which is not followed by a fall in blood sugar. This is well illustrated in Figures 2 and 3, representing a relatively small dose and a massive dose of both an insulin preparation and an "inactivated insulin" injected intravenously. All brands of American insulin thus tested revealed definite evidence of this hyperglycemic factor. However, there was considerable variation from lot to lot manufactured by any of the American companies; significant amounts were found in all crystalline insulins tested. Similar results were reported by Weisberg *et al.* (54).

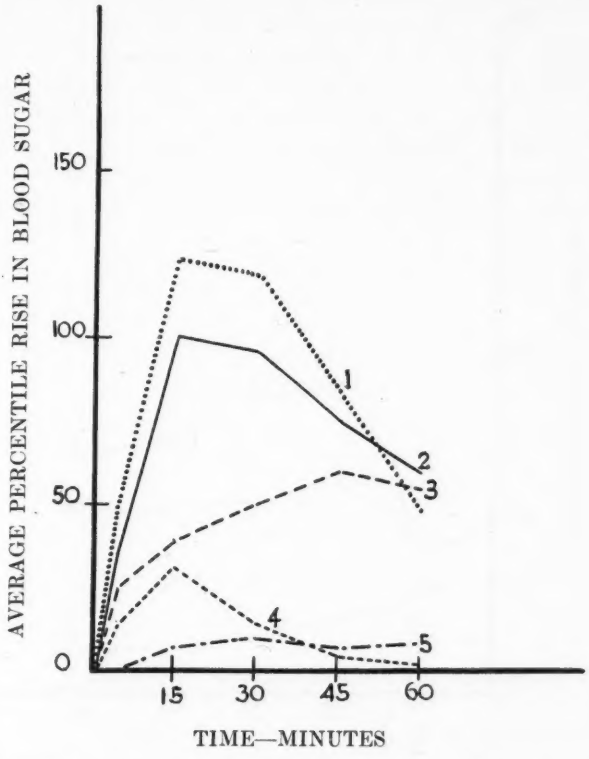

FIG. 5. Average blood sugar rise following the injection of inactivated insulin by different routes. 1. Intraportally (·····). 2. Intravenously (———). 3. Intraperitoneally (— — —). 4. Intramuscularly (- - - - -). 5. Subcutaneously (— · — · —).

A few experiments were performed with NOVO insulin. This material, presumably free of the glycogenolytic factor, did provoke a transient hyperglycemia when injected intravenously in massive dosage as is evidenced in Figure 4. On inactivation in the usual way it no longer provoked either hyperglycemia or hypoglycemia. Both insulin and the hyperglycemic factor were destroyed in this preparation by the inactivating method used.

3. Investigations were performed in a large number of animals on the effect of injecting inactivated insulin by different routes. Figure 5 illustrates graphically the results of these experiments. It was noted that in all cases the intraportal injection caused a more significant rise than by any other route although this is not so striking in the averages shown. When injected intravenously, a marked rise occurred; also a considerable rise followed intraperitoneal injection. The intramuscular injection produced

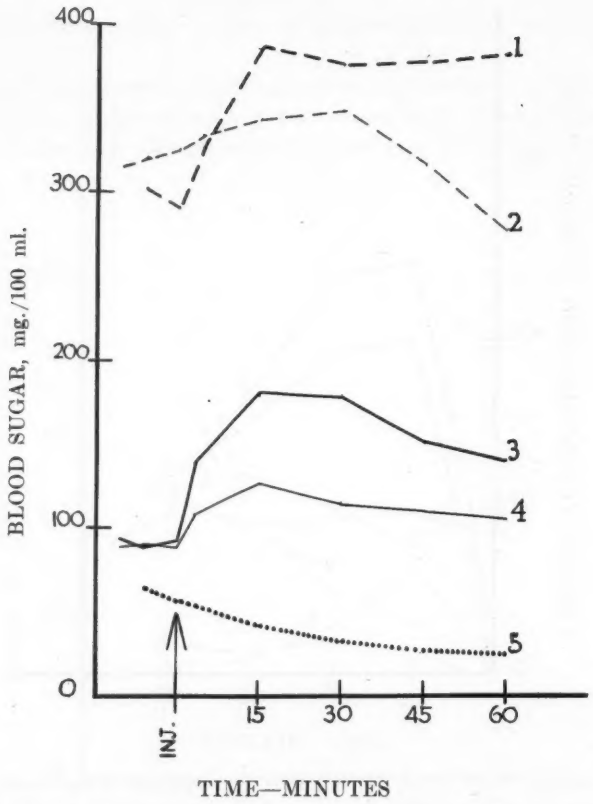

FIG. 6. Representative experiments showing blood sugar changes following intravenous injection of inactivated insulin. 1. Into a healthy pancreatectomized dog. (— — —). 2. Into a pancreatectomized dog untreated with insulin (- - -). 3. Into a normal well-fed animal (———). 4. Into a dog after fasting seventy-two hours (———). 5. Following evisceration and hepatectomy (·····).

a definite but moderate response, whereas after subcutaneous injection of equivalent amounts of the hyperglycemic factor (prepared from 240 units of a commercial insulin preparation) either no response was elicited or the rise was not significant. Hence the effect of this factor is not evident when insulin is administered by the usual route.

4. The effect of intravenous injection of inactivated insulin under various conditions was studied. Illustrative experiments are presented in Figure 6. It will be noted as previously reported by Zimmermann (48) that the pancreatectomized animal responds to this material when in good condition and well maintained on insulin. However, we noted that after such

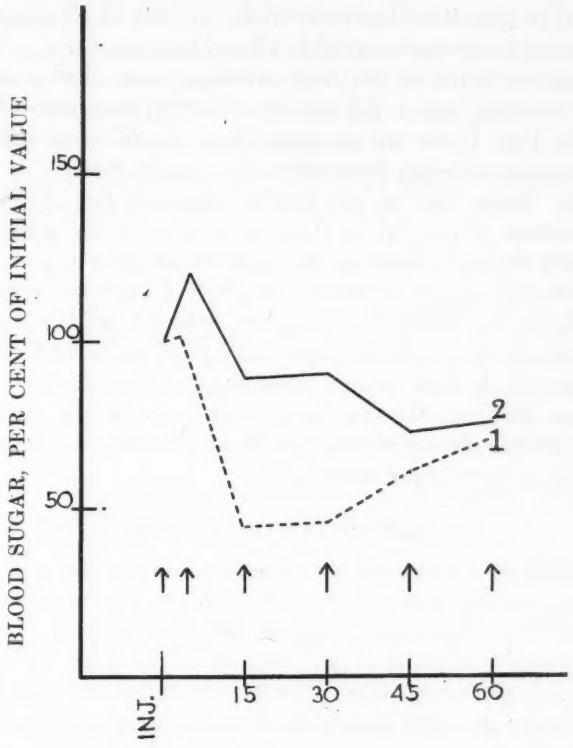

FIG. 7. Blood sugar changes following intravenous injections of small doses of insulin. 1. One unit of insulin (- - - - -). 2. One unit of insulin followed by repeated injections of inactivated insulin (———).

animals had been deprived of insulin, only minimal hyperglycemia resulted. Similarly, in contrast to well fed, normal animals, following prolonged fast the intact starved dog to which this agent was administered showed either no response or a greatly decreased elevation of blood sugar. After evisceration and hepatectomy the intravenous injection of inactivated insulin failed to alter the steady downward course of the blood sugar.

5. Figure 7 illustrates the ability of this material to counteract the hypoglycemic effect of insulin. When small doses of insulin are administered intravenously it is possible by repeated injection of "inactive" insulin either to prevent or greatly reduce the usual hypoglycemia.

6. A number of miscellaneous studies have been performed, several of which may be mentioned here. It has been found repeatedly that when

administered in quantities sufficient to elevate the blood sugar definitely, the hyperglycemic substance failed to affect blood pressure.

Glycogenolytic material has been obtained from the duodenum; this factor was therefore tested in 2 pancreatic fistula dogs and in 2 acute experiments on dogs. It did not provoke a flow of pancreatic juice in any of these experiments, although hyperglycemia was produced.

Studies by Rupp (55) on the insulin tolerance test in patients with Addison's disease show that in these patients primary transient hyperglycemia may develop following the intravenous injection of insulin. We have had the opportunity of testing the effect of the hyperglycemia factor in one patient with Addison's disease maintained satisfactorily with desoxycorticosterone acetate and salt and in a second patient with diabetes and Addison's disease. In both cases a blood sugar elevation which was normal in degree was obtained. When administered to patients in quantities sufficient to elevate the glucose level of the blood definitely, no significant effect was produced on circulating eosinophils.

SUMMARY AND CONCLUSIONS

1. The observations of many previous investigators have been affirmed, namely, that material can be obtained from the pancreas which elevates blood sugar. Our observations suggest that the primary, if not the sole means by which this material produces its effect is by the liberation of sugar from hepatic stores, since after hepatectomy and under conditions with low hepatic glycogen levels, such as starvation or uncontrolled diabetes following pancreatectomy, this material is relatively ineffective in producing hyperglycemia.

2. The hyperglycemic agent has been demonstrated to be most effective when administered intravenously, particularly by intraportal injection, and either ineffective or minimally effective after subcutaneous injection.

3. Although direct proof is lacking, indirect observations suggest that this material in order to be effective does not require the adrenal gland. Further observations are being made.

4. Clinical and experimental observations which have been discussed suggest that the hyperglycemic-glycogenolytic substance is an alpha cell hormone and its absence or presence in excess amounts may play a role in certain diseases.

Acknowledgments

I am indebted to Dr. S. A. Komarov of the S. S. Fels Foundation, Temple University School of Medicine, who prepared the extracts used in these studies and assisted in the initial experiments performed. I am also indebted to Mr. Bair and Dr. Hines of E. R. Squibb and Company, and to Dr. Barnes of Sharp and Dohme for generous supplies of insulin, and to Miss J. Hurwitz and Miss R. Pearl for technical assistance.

Addendum

Since this paper was submitted, McQuarrie, I.; Zimmermann, B., and Wright, W. S. have reported on "Deficiency of alpha cells of the pancreas as a possible etiological factor in familial hypoglycemias" in *Fed. Proc.* **9**: 337, 1950.

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The 1950 Meeting of the Association for the Study of Internal Secretions

The Thirty-Second Annual Meeting of The Association for the Study of Internal Secretions will be held at the Sir Francis Drake Hotel, Friday and Saturday, June 23 and 24, 1950, in San Francisco, California.

The Committee on Local Arrangements is comprised of Dr. Hans Lisser, Chairman, and Drs. Leslie L. Bennett, Roberto F. Escamilla, Minnie B. Goldberg, Gilbert S. Gordan, and Laurance W. Kinsell.

Hotel accommodations will be difficult to secure on short notice; therefore, members are urged to make their reservations at once. All requests must be addressed to: Dr. William Howard Rustad, American Medical Association (Hotel Committee), Room 200, Civic Auditorium, San Francisco, California.

The scientific sessions will be held in the Empire Room of the Sir Francis Drake, and registration will be in the French Room on the same floor. The annual dinner will be held in the Empire Room on Friday, June 23, at 7:30 P.M., preceded by cocktails at 6:30 P.M. in the French Room.

PROGRAM

FRIDAY, JUNE 23, 1950

8:30 A.M. Registration—French Room

I. 9:30 A.M. Empire Room

E. H. Rynearson, presiding

1. The Effect of Hypophysectomy and Growth Hormone on Phosphorus Metabolism. Irving I. Geschwind (by invitation), Choh Hao Li, and Herbert M. Evans (by invitation).
2. Studies on the Effects of Hypophysectomy and Adrenocorticotrophic Hormones on Certain Phosphorus Fractions in the Rat Liver. Irving I. Geschwind (introduced by Choh Hao Li).
3. The Effect of Growth Hormone on Hepatic Catalase, Erythrocyte Volume, and Hemoglobin in Rats. O. H. Gaebler, and James C. Mathies (by invitation).

4. The Effects of Prolonged Perfusion of the Dog's Pancreas with Glucose. Ernest M. Brown, Jr. (by invitation), F. C. Dohan, L. R. Freedman (by invitation), P. DeMoor (by invitation), and F. D. W. Lukens.

5. Action of Steroids on Cerebral Metabolism. R. C. Bentinck (by invitation), Gilbert S. Gordan, John E. Adams (by invitation), L. H. Arnstein (by invitation), and T. B. Leake (by invitation).

6. Hormonal Factors Affecting Avidin Secretion and Biotin Blood Levels in the Chick. Roy Hertz, W. W. Tullner (by invitation), and F. G. Dhyse (by invitation).

7. A Study of the Metabolism of Single Therapeutic Doses (2.0 mg.) of Estrone in Healthy Women in the Preovulatory and Postovulatory Phases of the Menstrual Cycle. Benjamin F. Stimmel, and Clair L. Stealy.

8. A Steroid which Promotes Tissue Growth without Concomitant Genital Activity. Gilbert S. Gordan, Eugene Eisenberg (by invitation), and Henry D. Moon (by invitation).

9. Factors in Wheat Germ Oil Affecting the Sex Organs of Hypophysectomized Rats. Ezra Levin, John F. Burns, A. Kaplan, and V. K. Collins (introduced by Carl R. Moore).

10. The Use of Progesterone in Treatment of Cancer of the Prostate. J. B. Trunnell, B. J. Duffy, Jr. (by invitation), Victor Marshall (by invitation), Willet Whitmore (by invitation), and Helen Q. Woodard (by invitation).

11. Tracer Studies with Labelled Preparations of Anterior Pituitary Hormones: *ACTH*. Martin Sonenberg, Albert S. Keston, and William L. Money (introduced by Rulon W. Rawson).

12. Fate of Intravenously Administered Adrenocorticotrophic Hormone (*ACTH*). John B. Richards (by invitation), Marshall Merkin (by invitation), Chi Ping Cheng (by invitation), and George Sayers.

13. The Effect of *ACTH* upon the Level of 17-Hydroxycorticosterone in the Adrenal Vein Blood of Dogs. Don H. Nelson (by invitation), Hans Reich (by invitation), and L. T. Samuels.

II. 2:00 P.M. Empire Room

E. A. Doisy, presiding

14. The Functional Relationship between the Pituitary Gland and the Parathyroids. Nils Tornblom (introduced by Fuller Albright).

15. Radioiodine (I^{131}) and Gonadal Function: An Experimental and Clinical Study. B. J. Duffy, Jr. (by invitation), J. T. Godwin (by invitation), J. B. Trunnell, and R. W. Rawson.

16. Carrier-Free Radioactive Iodine (I^{131}) Thyroid Uptake and Urinary Excretion in Normal and Hypothyroid Children. Wm. A. Reilly, and Dina L. Bayer (introduced by H. Lisser).

17. Uptake of Radioactive Iodine (I^{131}) by the Thyroid Gland after Intravenous Administration of Tracer Doses. Joseph P. Kriss.

18. Observations on the Relationship between Bone Marrow Activity and Thyroid Function. Arnold R. Axelrod, and Lawrence Berman (introduced by William O. Maddock).

19. Thyroxine-Like Activity of Acrylic Acid Analogues of Thyroxine. S. B. Barker, H. M. Klitgaard (by invitation), H. B. Dirks, Jr. (by invitation), S. Wawzonek (by invitation), and S. C. Wang (by invitation).

20. Clinical Application of Certain Mercaptoimidazoles to Hyperthyroidism. Thomas

H. McGavack, Jacqueline Chevalley (by invitation), A. M. Shearman (by invitation), I. J. Drekter, and Sidney Stern (by invitation).

21. Thyroid Hormone and Tissue Cholesterol Distribution. Walter Marx, Lore Marx, and Samuel B. Weiss (introduced by H. J. Duel, Jr.).

22. Studies on Hormonal Control of Serum Lipid Partition in Man. David Adlersberg, Louis E. Schaefer (by invitation), and Rhoda Dritch (by invitation).

23. The Effects of *ACTH*, of Cortisone, and of Other Steroid Compounds upon Fat Metabolism in Diabetic and Non-diabetic Human Subjects. Laurance Kinsell, Sheldon Margen, George Michaels (by invitation), Robert Reiss (by invitation), Robert Frantz (by invitation), and John Carbone (by invitation). With the technical assistance of Gerald Liebert, Carl Anderson, Lila Suiter, and Maxine E. Hutchin.

24. The Anabolic Effects of Testosterone Compound as Manifested by Increased Height and Weight Gain in Boys. H. Lisser, and Gilbert S. Gordan.

25. The Effect of Testosterone Administration upon the Human Testis. Carl G. Heller, Warren O. Nelson, Irvin C. Hill (by invitation), Edward Henderson, William O. Maddock, and E. C. Jungck.

26. Sex-Linked Growth Retardation and Juvenile Diabetes Mellitus. Robert R. Commons (by invitation), Oner C. Barker (by invitation), and E. Kost Shelton.

27. Pituitary Infantilism. Treatment with Purified Growth Hormone, Thyroid, and Sublingual Methyl Testosterone. Roberto F. Escamilla, and Leslie Bennett.

III. Annual Dinner, Friday, June 23, 1950

6:30 P.M., Cocktails, French Room
7:30 P.M., Dinner, Empire Room
President E. A. Doisy, presiding

PRESENTATION OF: E. R. Squibb & Sons Award for 1950, Ciba Award for 1950, Ayerst, McKenna & Harrison Fellowship for 1950, Schering Fellowship for 1950.
By *E. C. Kendall*, Chairman of Awards Committee for 1949–50

PRESIDENT'S ADDRESS: DR. DOISY.

SATURDAY, JUNE 24, 1950

IV. 9:00 A.M. Empire Room

J. S. L. Browne, presiding

28. Familial Female Pseudohermaphroditism. R. C. Bentinck (by invitation), H. Lisser, Frank Hinman (by invitation), and Herbert C. Traut (by invitation).

29. The Effects on the Linear Growth, Weight Gain, and Histologic Pattern of the Testes of Young Rats by Large Doses of *DOCA*, Lipoadrenal Cortex, Thyroid, Thyroxin, Testosterone Propionate and Physiologic Doses of Testosterone Propionate. Morton J. Nyda and Gerson R. Biskind.

30. The Relation of Adrenal Activity to Experimental Hypertension. Lena A. Lewis, and Irvin H. Page.

31. Interrelations between the Adrenal Cortex and Lymphoidal Tissues: Growth of Adrenal Transplants in Spleen and Thymus with Local Thymic Atrophy. Edward J. Bomze (by invitation), and Nathan B. Friedman.

32. The Effect of Adrenal Transplanting on the Stress Reaction to Hypoxia. L. L. Langley (introduced by Robert S. Teague).

33. The Use of Cystine Labeled with S^{35} as a Tool for Studying Adrenal Physiology and Protein Metabolism. Norman D. Lee (by invitation), and Robert H. Williams.

34. Changes in Responsiveness to Insulin in Diabetes Mellitus Induced by the Administration of Desoxycorticosterone. Hyman J. Zimmerman (by invitation), Alvin E. Parish (by invitation), and Louis K. Alpert.

35. The Metabolic Effects of *ACTH* in a Patient with Cushing's Syndrome and Acromegaly. Leonard P. Eliel, and Olof H. Pearson (introduced by Konrad Dobriner).

36. Effect of Adrenocorticotrophic Hormone (*ACTH*) on the Pathological Changes Induced by Desoxycorticosterone Acetate (*DCA*). Charles A. Rosenberg (by invitation), Dixon M. Woodbury (by invitation), and George Sayers.

37. The Response of the Adrenal Cortex and Thyroid Gland and Cortisone in Patients with Hypothyroidism and the Nephrotic Syndrome. S. R. Hill, Jr. (by invitation), P. H. Forsham (by invitation), M. Roche (by invitation), and G. W. Thorn.

38. The Effect of Small Dosages of *ACTH* Protein and *ACTH* Peptides in Cases of Rheumatoid Arthritis. Rolf Luft, Bjorn Sjogren (by invitation), and Choh Hao Li.

39. Erythrocyte Sedimentation Rate in DCA and Cortisone Treated Animals. Hans Selye, and Niall Carey (by invitation).

40. Oral Steroid Administration in Rheumatoid Arthritis. Harry Freeman (by invitation), and Gregory Pincus.

Conjoined Program with
American Diabetes Association

V. 2:00 P.M. Empire Room

Howard F. Root and E. A. Doisy, presiding

41. Growth Hormone and Experimental Diabetes. F. G. Young.

42. *ACTH* Diabetes in Man. The End Organ Response Versus the Adrenocortical Response. Jerome W. Conn.

43. Studies on Carbohydrate Metabolism in Adrenal Cortical Hypersecretion. P. H. Forsham (by invitation), G. W. Thorn, T. F. Frawley (by invitation), and D. L. Wilson (by invitation).

44. Modification of the Metabolic Effects of Adrenocorticotrophic Hormone and Growth Hormone by the Administration of a Diet High in Potassium Chloride. Leslie L. Bennett, John E. Whitney (by invitation), and Constantine Glafkides (by invitation).

45. Observations on the Pathogenesis of Cushing's Syndrome. Randall G. Sprague, Harold L. Mason, and Marschelle H. Power (by invitation).

46. Pathways of Corticosterone Synthesis. O. Hechter, B. P. Jacobsen (by invitation), R. Jeanloz (by invitation), H. Levy (by invitation), G. Pincus, and V. Schenker (by invitation).

47. The Islets of Langerhans in Man Visualized by Phase Contrast Microscopy. W. Stanley Hartroft (by invitation).

48. The Incidence and Forms of Vascular Disease in Diabetes. E. T. Bell (by invitation).

4:00–4:30 P.M.—General Discussion

VI. 4:30 P.M. Annual Business Meeting

TO BE READ BY TITLE

49. The Effect of Desoxycorticosterone Acetate (*DCA*) on Blood Pressure and Renal Function in Human Subjects. Rolf Luft, and Bjorn Sjogren (by invitation).

50. Studies on Circulating Growth Hormone. Francis S. Greenspan (introduced by Choh Hao Li).

51. Estrogenic Interruption of Broodiness in the Fowl. R. George Jaap, and Edward F. Godrey (by invitation).

52. Response to Estrone of Intraocular Endometrial Implants of Littermates in Female Rabbits with Uteri Intact. Elly M. Jacobsen (introduced by Clara M. Szego).

53. Quantitative Studies on the Production of Pseudopregnancy by Estrogens. Albert Segaloff.

54. The Agreement of Clinical Diagnosis with Vaginal Smears, as Shown by the Evaluation of Symptoms, Colpocytogram, and Glycogen Index. Manual M. Villaverde.

55. The Effect of Steroid Hormones Applied Directly to Senile Human Skin. Joseph W. Goldzieher, William B. Rawls (by invitation), Irene S. Roberts (by invitation), and Max A. Goldzieher.

56. Oral Administration of Wheat Germ Oil Concentrate as an Aid in the Reduction of Pregnancy Complications. Wynne M. Silbernagel, and James B. Patterson (by invitation).

57. The Role of the Liver and Kidney in the Metabolism of Intravenous Testosterone by Human Subjects. C. D. West (by invitation), Harold Brown (by invitation), Frank H. Tyler, and L. T. Samuels.

58. Frequency of Retarded Bone Age in a Group of Enuretic Children. R. M. Archibald.

59. Clinical Experience with the Use of Cortisone Pellets in the Treatment of Adrenal Cortical Insufficiency. G. W. Thorn, P. H. Forsham (by invitation), T. F. Frawley (by invitation), and S. R. Hill, Jr. (by invitation).

60. A Study of Adrenal Cortical Response in Health and Disease. The 48-Hour *ACTH* Test. M. Roche (by invitation), P. H. Forsham (by invitation), C. C. Forsham (by invitation), and G. W. Thorn.

61. The Effects of *ACTH*, and of Steroid Compounds upon Ketolysis in Diabetic and Non-Diabetic Human Subjects. Sheldon Margen, George Michaels (by invitation), Charles Mudrick (by invitation), Harry Barton (by invitation), Robert Reiss, and Laurance Kinsell. With the technical assistance of Gerald Liebert, Carl Anderson, Lila Suiter, Maxine Hutchin, and Don Myers.

62. Administration and Subsequent Withdrawal of *ACTH* Studied Simultaneously in a Gouty Father and in His Hyperuricemic Son. W. Q. Wolfson, C. Cohn, C. Shore (by invitation), W. Wise (by invitation), and S. Sideman (by invitation).

63. Increased Adrenal Cortical Function Accompanying Prolonged Remission Induced by *ACTH* in Myasthenia Gravis. B. Katz (by invitation), M. Katz (by invitation), W. Q. Wolfson, C. Cohn, and B. B. Rubenstein.

64. Adactar and Adactar-Gelatins Pituitary Adrenocorticotrophin Preparations with Prolonged Activity. W. Q. Wolfson, R. E. Thompson, and C. Cohn.

65. The Effect of Crude Anterior Pituitary Preparation on Haemopoiesis in Normal, Thyroidectomized and Gonadectomized Rats. Niall Carey (introduced by Hans Selye).

66. Thyroid Extract Versus Thyroxine. Critical Evaluation with Particular Reference to Functional Infertility. A. R. Abarbanel.

67. Thyrotoxin Neuropathy. Max A. Goldzieher, Thomas H. McGavack, and Joseph W. Goldzieher.

68. The Treatment with Desiccated Thyroid of Periodic Disturbances with Menses in Mentally Defective Patients. Meyer M. Harris, and Theodora M. Abel (by invitation).

69. Hypopituitary Myxedema, A Non-Cachectic Form of Simmonds' Disease: Report of Five Cases Emphasizing the Significance of Plasma Cholesterol Levels. Leela S. Craig, Roberto F. Escamilla, and Gilbert S. Gordan.

70. Cushing's Syndrome from Adrenal Cortical Tumor with Special Reference to the Surgical Mortality: A Report of Three Cases and Review of the Literature. M. B. Goldberg, Gilbert S. Gordan, William C. Deamer (by invitation), Frank Hinman, Jr. (by invitation), and Elliot Rapaport (by invitation).

71. Growth and Maturation in a Case of Idiopathic Precocious Puberty. Leona M. Bayer.

72. Familial Insulin-Resistant Diabetes Mellitus. Myron S. Arrick (introduced by E. A. Doisy).

73. Adjuvant Use of Pregnenolone in Seminal Inadequacy. A. R. Abarbanel.

74. A Simple Method for the Determination of Neutral 17-Ketosteroids Based on a Novel Means for the Removal of Interfering Pigments. I. J. Drekter, Alexander Heisler (by invitation), and Thomas H. McGavack.

75. Studies with Pregnenolone: 1. Nitrogen and Electrolytic Balance. Jonas Weissberg (by invitation), Jacqueline Chevalley (by invitation), and Thomas H. McGavack.

76. Studies with Pregnenolone: 2. Clinical Applications. Thomas M. McGavack, Jacqueline Chevalley (by invitation), and Jonas Weissberg (by invitation).

77. The Effect of Female Hormones on the Basal Body Temperature. M. James Whitelaw.

78. The Treatment of Juvenile Acne with Topical Estrogens. M. James Whitelaw.

79. Acetoxypregnenolone. George Joyce Hall.

BOOKS RECEIVED

THE following list acknowledges receipt of books which it has not been possible for us to review as yet:

American Lectures in Endocrinology. Edited by WILLARD O. THOMPSON, M.D., Clinical Professor of Medicine, University of Illinois College of Medicine, Chicago, Illinois. Charles C Thomas, Publisher, Springfield, Illinois.

Chemical Developments in Thyroidology. By WILLIAM T. SALTER, M.D., Professor of Pharmacology, Yale University School of Medicine, New Haven, Connecticut; 93 pages; 6 illustrations; 119 references; 1950. Price $2.00.

Diagnosis and Therapy of Gynecological Endocrine Disorders. By C. L. BUXTON, M.D., Med. Sc.D., Associate Professor of Clinical Obstetrics and Gynecology, Columbia University College of Physicians and Surgeons, Associate Attending Obstetrician and Gynecologist, Sloane Hospital for Women, New York City, and E. T. ENGLE, Ph.D., Professor of Anatomy, Columbia University College of Physicians and Surgeons, New York City; 70 pages; 1949. Price $2.00.

The Diagnosis and Treatment of Adrenal Insufficiency. By GEORGE W. THORN, M.D., Hersey Professor of the Theory and Practice of Physic, Harvard Medical School, and Physician-in-Chief, Peter Bent Brigham Hospital, Boston, Massachusetts, with the collaboration of PETER H. FORSHAM, M.D., M.A. (Cantab.), Assistant in Medicine, Harvard Medical School and Peter Bent Brigham Hospital, Boston, Massachusetts, and KENDALL EMERSON, JR., M.D., Associate in Medicine, Harvard Medical School and Senior Associate in Medicine, Peter Bent Brigham Hospital, Boston, Massachusetts; 180 pages; 25 tables; 32 illustrations; 121 references; 1949. Price $5.50.

Embryonic Sex Hormones and Sexual Differentiation. By CARL R. MOORE, Ph.D., Professor of Zoology, The University of Chicago, Chicago, Illinois; 87 pages; 22 figures; 145 references; 1947. Price $2.00.

The Endocrines in Diabetes. By SAMUEL SOSKIN, M.D., Director of the Research Institute, and Medical Director, Michael Reese Hospital; Professorial Lecturer in Physiology, University of Chicago, Chicago, Illinois; 30 pages; 9 figures; 1948. Price $0.90.

The Function of the Thyroid Gland. By J. H. MEANS, M.D., Jackson Professor of Clinical Medicine, Harvard University; Chief of Medical Services, Massachusetts General Hospital, Boston, Massachusetts; 42 pages; 6 figures; 1949. Price $0.90.

The Management of the Climacteric (Male or Female). By ELMER L. SEVRINGHAUS, M.D., Director of Endocrine and Nutritional Clinics, Gouverneur Hospital, New York, N. Y; 35 pages; 1948. Price $1.35.

Nutrition and Hormones. By LEO T. SAMUELS, Ph.D., Professor of Biochemistry, University of Utah School of Medicine, Salt Lake City, Utah; 56 pages; 9 figures; 10 tables; 118 references; 1948. Price $1.65.

Obesity. By EDWARD H. RYNEARSON, M.D., Associate Professor of Medicine, Mayo Foundation, and CLIFFORD F. GASTINEAU, M.D., Fellow in Medicine, Mayo Foundation; 146 pages; 18 tables; 9 illustrations; 422 references; 1949. Price $3.50.

The Present Status of the Surgical Treatment of Hyperthyroidism. By WARREN H. COLE,

M.D., Professor and Head of the Department of Surgery, University of Illinois College of Medicine, Chicago, Illinois; and EDSON F. FOWLER, M.D., Assistant in Surgery, University of Illinois College of Medicine, Chicago, Illinois and Associate Surgeon, St. Francis Hospital, Evanston, Illinois; 94 pages; 16 figures; 5 tables; 1948. Price $2.00.

Simmonds' Disease: Extreme Insufficiency of the Adrenohypophysis By R. F. FARQUHARSON, M. B., Professor of Medicine and Head of the Department, University of Toronto; and Physician-in-Chief, Toronto General Hospital, Toronto, Canada; 102 pages; 7 tables; 5 illustrations; 164 references; 1950. Price $2.00.

Brookhaven Conference Report—Conference on Biological Applications of Nuclear Physics, July 12–27, 1948; 159 pages; 32 tables; 67 illustrations. Brookhaven National Laboratory, Associated Universities, Inc., Upton, New York. Price $1.50.

Coeficiente Citológico y Correlación Hipofisotiroidea. By ESTANISLAO DEL CONTE, Doctor en Medicina, Jefe de la Sección Histofisiología del Instituto de Anatomía General y Embriología de la Facultad de Ciencias Médicas de Buenos Aires; 87 pages; 34 illustrations; 1949. El Ateneo, Buenos Aires, Argentina.

Conferences on Metabolic Aspects of Convalescence. Edited by EDWARD C. REIFENSTEIN, JR., M.D., Sloan-Kettering Institute, New York, N. Y. Transactions of the Sixteenth Meeting, New York, N. Y., October 27–28, 1947; 168 pages. Publication of Josiah Macy, Jr. Foundation, New York, N. Y., price $3.00: and Transactions of the Seventeenth Meeting, New York, N. Y., March 29–30, 1948, 246 pages, price $4.00.

Congenital Anomalies of the Heart and Great Vessels. (Clinicopathologic Study of 132 Cases.) By THOMAS J. DRY, M.B., Ch. B., M.S. in Medicine, Associate Professor of Medicine; JESSE E. EDWARDS, B.S., M.D., Assistant Professor of Pathologic Anatomy; ROBERT L. PARKER, M.S. in Medicine, M.D., Assistant Professor of Medicine; HOWARD B. BURCHELL, M.D., Ph.D. in Medicine, Assistant Professor of Medicine (all of the Mayo Foundation for Medical Education and Research, Graduate School, Univ. of Minnesota, and Consulting Physicians, Mayo Clinic); H. MILTON ROGERS, B.S., M.S. in Medicine, M.D., Fellow in Medicine, Mayo Foundation; ARTHUR H. BULBULIAN, M.S., D.D.S., Director, Museum of Hygiene and Medicine, Mayo Foundation for Medical Education and Research; 83 pages; 96 illustrations; 1949. Charles C Thomas, Publisher, Springfield, Illinois. Price $4.50.

La Différenciation du Sexe et l'Intersexualité chez les Vertébrés. By KITTY PONSE, Professeur Extraordinaire à l'Université de Genève; 366 pages; 170 illustrations; 1949. F. Rouge & Cie, S. A., Librairie de l'Université, Lausanne.

Endocrinologia. Ed. 2. By DIONISIO M. GONZALEZ TORRES, Professor Suplente da Faculdade de Medicina de Asunción, Paraguay; Chefe da Secção de Endocrinologia do Laboratório Paulista de Biologia; Assistente Voluntário no Ambulatório de Glândulas Endócrinas da Santa Casa de Misericórdia de São Paulo; 759 pages; 148 figures; 1947. Edigraf, Ltda., São Paulo, Brasil.

Enfermedades del Tiroides. By P. PIULACHS, Catedrático de Patología Quirúrgica de la Facultad de Medicina de Barcelona, del Consejo Superior de Investigaciones Científicas; and J. MA. CAÑADELL, Director del Departamento de Endocrinología (Clínica Médica B), del Consejo Superior de Investigaciones Científicas; 600 pages; 315 illustrations; 6 tables; 1950. Publicaciones Médicas, José Janés, editor, Barcelona.

The Eosinophil Count in Health and in Mental Disease: a Biometrical Study. By FINN RUD, from Universitetets Psykiatriske Institutt, Psykiatrisk Klinikk, Vinderen Pr. Oslo, Norway. Translated from Norwegian by LEKTOR OSKAR SIK; 443 pages; 66 figures; 54 tables; 1947. Johan Grundt Tanum Forlag, Oslo, Norway. Price, Norwegian kroner 20.00. Postage, 2.15.

Die Erkrankungen der Endokrinen Drüsen. By Prof. Dr. Med. et Phil. ADOLF OSWALD, Zurich, Switzerland; 589 pages; 44 illustrations; 1949. Medizinischer Verlag Hans Huber, Bern, Switzerland. Sfr. 58.

Essentials of Gynecologic Endocrinology. By GARDNER M. RILEY, Ph.D., Assistant Professor of Obstetrics and Gynecology, University of Michigan Medical School; 215 pages; 1948. Caduceus Press, Ann Arbor, Michigan.

Factors Regulating Blood Pressure. Transactions of the Third Conference, May 5–6, 1949, New York. Edited by B. W. ZWEIFACH and EPHRAIM SHORR, Dept. of Medicine, Cornell Univ. Medical College, New York; 280 pages, 1950. Publication of Josiah Macy, Jr. Foundation, New York, N.Y. Price $2.55.

Female Sex Endocrinology. By CHARLES H. BIRNBERG, M.D. Associated with the Department of Female Sex Endocrinology, Jewish Hospital, Brooklyn, N. Y.; 142 pages; 30 illustrations; 1949. J. B. Lippincott Company, Philadelphia. Price $4.00.

Fertility in Marriage: a Guide for the Childless. By LOUIS PORTNOY, M.D. and JULES SALTMAN; A medical book for the layman; 265 pages. Farrar, Straus & Company, Inc., New York, N. Y. Price $3.00.

Haemolytic Disease of the Newborn. By M. M. PICKLES, D.M., Nuffield Graduate Assistant in Clinical Pathology, Radcliffe Infirmary, Oxford, England; 191 pages; 21 illustrations; 19 tables; 1949. Charles C Thomas, Publisher, Springfield, Illinois. Price $4.50.

Helpful Hints to the Diabetic. By WILLIAM S. COLLENS, B.S., M.D., Chief of the Diabetic Clinic and of the Clinic for Peripheral Vascular Diseases, and Associate Attending Physician, Maimonides Hospital; Attending Physician, Metabolic Diseases, Jewish Sanitarium and Hospital for Chronic Diseases, Brooklyn, N. Y.; and LOUIS C. BOAS, A.B., M.D., Chief of the Diabetic Clinic and Associate Visiting Physician, Greenpoint Hospital; Associate Physician, Metabolic Diseases, Jewish Sanitarium and Hospital for Chronic Diseases, Assistant Physician, Maimonides Hospital, Brooklyn, N. Y.; 135 pages; 64 illustrations; 21 tables; 1949. Charles C Thomas, Publisher, Springfield, Illinois. Price $3.00.

Het Onderzoek Bij Stoornissen in de Mannelijke Vruchtbaarheid. By GERHARDUS HELLINGA, University of Amsterdam; 328 pages; 39 tables; 1949. N. V. Noord-Hollandsche Uitgevers Maatschappij, Amsterdam, The Netherlands. Price $9.50.

Hormones in Clinical Practice. By H. E. NIEBURGS, M.D., Research Associate Department of Endocrinology, and Assistant Professor, Department of Oncology, University of Georgia; lately Research Endocrinologist, Department of Obstetrics and Gynaecology, British Post-Graduate Medical School, and Clinical Assistant, Endocrine Clinic, Westminster Hospital, London; 400 pages; 57 illustrations; 1950. Paul B. Hoeber, Inc., New York, N. Y. Price $5.50.

Human Fertility and Problems of the Male. By EDMOND J. FARRIS, Ph.D., Executive Director, Associate Member, The Wistar Institute of Anatomy and Biology, Philadelphia, Pa.; 227 pages; 33 illustrations; 3 plates; 40 tables. The Author's Press, Inc., White Plains, N. Y.

Liver Injury. Transactions of the Eighth Conference, April 28–29, 1949, New York. Edited by F. W. HOFFBAUER, M.D., Dept. of Medicine, Univ. of Minnesota

Medical School, Minneapolis, Minn.; 164 pages; 1950. Publication of Josiah Macy, Jr. Foundation, New York, N.Y. Price $1.60.

Medical Research in France during the War. Thirty articles (in English) gathered and presented by JEAN HAMBURGER, Professeur agrégé à la Faculté de Médecine; Médecin des Hôpitaux de Paris. Foreword by PROFESSEUR PASTEUR VALLERY-RADOT, Membre de l'Institut; 306 pages; 1947. Éditions Médicales, Flammarion et Cie.

Menstruation and Its Disorders. Proceedings of the Conference held under the auspices of The National Committee on Maternal Health, edited by EARL T. ENGLE, Ph.D., College of Physicians and Surgeons, New York; 366 pages; 19 tables; 95 figures; 34 graphs; 1950. Charles C Thomas, Publisher, Springfield, Illinois. Price $6.50.

Neurology. Ed. 4. By ROY R. GRINKER, M.D., Director of The Institute for Psychosomatic and Psychiatric Research and Training, and Chairman of The Department of Neuropsychiatry, Michael Reese Hospital, Chicago; and PAUL C. BUCY, M.D., Professor of Neurology and Neurological Surgery, University of Illinois College of Medicine, Chicago, Illinois; 1145 pages; 416 figures; 1949. Charles C Thomas, Publisher, Springfield, Illinois.

Ocular Therapeutics. By WILLIAM J. HARRISON, Phar. D., M.D., Associate Professor of Ophthalmology, Jefferson Medical College; Chief of the Ophthalmological Clinic, Jefferson Hospital; Attending Surgeon, Willis Hospital, Philadelphia; 122 pages; 1947. Charles C Thomas, Publisher, Springfield, Illinois. Price $3.50.

Physiology in Diseases of the Heart and Lungs. Harvard University Monograph in Medicine and Public Health, Number 10. By MARK D. ALTSCHULE, Assistant Professor of Medicine, Harvard Medical School; Visiting Physician and Research Associate, Beth Israel Hospital; Director of Internal Medicine and of Research in Clinical Physiology, McLean Hospital; 383 pages; 1949. Harvard University Press, Cambridge, Massachusetts. Price $5.00.

Physiology of the Uterus. Ed. 2. S. R. M. REYNOLDS, M.A., Ph.D., Staff Member, Physiologist, Department of Embryology, Carnegie Institution of Washington; Lecturer in Obstetrics, Johns Hopkins University School of Medicine, Baltimore, Md.; 633 pages; 67 illustrations; 1949. Paul B. Hoeber, Inc., New York, N. Y. Price $12.50.

Practical Neurological Diagnosis. Ed. 4. By R. GLEN SPURLING, M.D., Clinical Professor of Surgery (Neurosurgery), University of Louisville School of Medicine, Louisville Kentucky; 290 pages; 118 illustrations; 1950. Charles C Thomas, Publisher, Springfield, Illinois. Price $5.00.

Problems of Aging. Transactions of the Tenth and Eleventh Conferences, Feb. 9-10, 1948 and April 25–26, 1949, New York, N.Y. Edited by NATHAN W. SHOCK, Chief, Section on Gerontology, National Heart Institute, and the Baltimore City Hospitals Baltimore, Md.; 258 pages, 1950. Publication of Josiah Macy, Jr. Foundation, New York, N.Y. Price $3.75.

Problems of Early Infancy. Transactions of the Second Conference, March 1–2, 1948, New York, N. Y. Edited by MILTON J. E. SENN, M.D., Departments of Pediatrics and Psychiatry, Yale University School of Medicine, New Haven, Conn.; 120 pages. Publication of Josiah Macy, Jr. Foundation, New York, N. Y. Price $1.00.

Radiographic Atlas of Skeletal Development of the Hand and Wrist. By WILLIAM WALTER GREULICH, M.A., Ph.D., Professor of Anatomy, Stanford University School of Medicine, formerly Professor of Physical Anthropology and Anatomy and Director of the Brush Foundation, Western Reserve University School of Medicine; and S.

IDELL PYLE, M.S., Research Associate, Brush Foundation and Department of Anatomy, Western Reserve University School of Medicine; 203 pages; 65 figures; 1950. Stanford University Press, Stanford, California. Price $10.00.

The Thyroid Hormones and Their Action. By G. MANSFELD, M.D., Professor of Physiology, University of Budapest. Translated by ERWIN PULAY, M.D.; 169 pages; 60 diagrams; 55 tables; 1949. Frederick Muller Ltd., London. Price 24/—net.

Trichomonas Vaginalis and Trichomoniasis. By RAY E. TRUSSELL, M.D., Associate in Hygiene and Preventive Medicine, former Research Assistant in Obstetrics and Gynecology, State University of Iowa. With an Introduction by E. D. PLASS, M.D., Professor of Obstetrics and Gynecology, State University of Iowa; 288 pages; 19 figures; 16 tables; bibliography of 1586 references; 1947. Charles C Thomas, Publisher, Springfield, Illinois. Price $6.00.